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Review

The Pivotal Role of the Membrane-Bound O-Acyltransferase Domain Containing 7 in Non-Alcoholic Fatty Liver Disease

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a common and prevalent disorder affecting 25 percent of the adults in the United States and 32 percent of adults globally. It is one of the common causes of chronic liver disease characterized by steatosis, which can lead to inflammation, fibrosis, and cirrhosis. NAFLD is strongly associated with obesity and insulin resistance. Multiple genetic variants have been consistently found to be associated with NAFLD; one of them is found in the TMC4-*MBOAT7* loci. One variant (rs641738 C>T) within *MBOAT7* encoding lysophosphatidyl inositol acyltransferase increases the risk for NAFLD development and triggers hepatic inflammation by regulating arachidonic acid levels. This review provides an overview of the *MBOAT7* gene, pathogenesis of NAFLD, understanding the regulation of *MBOAT7* and mechanistic link between *MBOAT7* and NAFLD. It further summarizes pathophysiologically relevant in vivo and in vitro studies on *MBOAT7* and challenges in treating complex NAFLD with recent progress made in the treatment of NAFLD. As such, this review provides useful information on *MBOAT7* and NAFLD interrelation, which has the potential of deciphering novel therapeutic targets rather than well-known genetic variants such as PNPLA3 and TM6SF2.

Keywords: non-alcoholic fatty liver disease; *MBOAT7*; lipid metabolism; hepatic steatosis; fibrosis; therapeutic targets; insulin resistance; pathogenesis

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1. Introduction

NAFLD is a complex, multifactorial disease encompassing a broad spectrum of disorders including hepatic steatosis, fibrosis, cirrhosis, and progression to hepatocellular carcinoma and is strongly associated with metabolic syndrome including diabetes mellitus, insulin resistance and obesity. Noteworthy, the total number of populations with non-alcoholic fatty liver disease (NAFLD) exceeds the combined population with obesity and diabetes mellitus globally [1]. In addition, multiple studies have established a strong association between NAFLD and increased risk of cardiovascular diseases and its complications. More importantly, NAFLD is recognized as an independent risk factor in cardiovascular diseases [2]. These diseases share multiple pathophysiological features and are closely interrelated in their progression.

According to histology, NAFLD is defined by the presence of hepatic lipid accumulation in >5% of hepatocytes [2]. NAFLD has been predicted to be the most frequent indication for liver transplantation by 2030 [3]. It further contributes to extra-hepatic chronic complications and regulatory pathways [4]. The current gender-based prevalence of NAFLD is approximately 30–40% in men and 15–20% in women [5].

Recent studies have identified several genetic susceptibility variants comprising of single-nucleotide polymorphisms in *PNPLA3* (rs738409), *TM6SF2* (rs8542926), and *LXPLAL1* (rs12137855) [6]. The contributions of the *PNPLA3* polymorphism in NAFLD have

been widely studied, while in contrast, the molecular biochemical mechanistic insights underlying the *MBOAT7* polymorphism are little understood [6]. Recently, the identification of genetic variant rs641738 near two genes encoding the *MBOAT7* gene and transmembrane channel-like 4 (TMC4) in determining the risk of NAFLD and its complications has gained utmost importance and still needs further exploration for a targeted drug design to treat complex NAFLD [7]. However, the available data presently suggest that TMC4 is not expressed abundantly in the human liver, and previous studies revealed that the CRISPR/Cas9-mediated TMC4 knockout in mice does not provoke hepatic steatosis [8]. More importantly, it has been reported that *MBOAT7* loss of function alone promotes liver disease progression via the accumulation of lysophosphatidylinositol lipids, thereby highlighting the unique role of *MBOAT7* in NAFLD pathogenesis [8].

MBOA7 is a lysophophosphatidylinositol (LPI) acyltransferase preferentially transferring polyunsaturated fatty acids (PUFAs) to LPI. Emerging genome-wide association studies reported that a genetic variant within the *MBOAT7* gene is closely related to non-alcoholic steatohepatitis (NASH), attributed to an increase in triglyceride synthesis through canonical and non-canonical pathways of de novo lipogenesis [9]. This review summarizes our knowledge of *MBOAT7* and NAFLD association, which potentially opens new avenues for drug design strategies to treat this multi-system disease. The selectivity of *MBOAT7* for long PUFAs such as arachidonic acids suggests that an abundance of specific phosphoinositols are regulated by *MBOAT7* without alterations in total phosphoinositol content [8].

2. *MBOAT7* Gene—An Overview

The *MBOAT7* gene, also known as lysophosphatidylinositol acyltransferase 1 (LPLAT1), is located on the long arm of human chromosome 19 (19q13.42) and encodes an enzyme with a size of 472 amino acids. The protein is highly expressed in liver, heart, testis, and adipose tissue, and has a molecular mass of 52,765 Da [10]. The *MBOAT7* protein is a highly conserved member of the membrane-bound O-acyltransferases family of integral membrane proteins composed of six transmembrane domains present on the endoplasmic reticulum, lipid droplets, and mitochondria-associated membranes (Figure 1) [10–12].

The encoded protein is a lysophosphatidylinositol acyltransferase having specificity for the arachidonoyl-CoA acyl donor, involved in reacylation of phospholipids (PLs) as a part of Land cycle (remodeling of PL) [9]. The lysophospholipid acyl transferase activity of *MBOAT7* preferentially incorporates arachidonic acid into PLs [9]. In the liver, *MBOAT7* participates in the regulation of triglyceride metabolism through the phosphatidylinositol acyl-chain remodeling regulation. *MBOAT7* plays a unique role in Land's cycle, in diversifying the fatty acid composition of membrane phosphatidylinositol (PI) species and not PLs with other head groups.

The two enzymatic reactions of the Land's cycle include diacylation of unsaturated PLs from the sn-2 position of PLs catalyzed by phospholipases and esterification of fatty acids to lysophospholipid catalyzed by acyltransferases resulting in the release of newly modeled PLs [11,12]. *MBOAT7* incorporates free PUFAs such as arachidonic acid into lysophospholipids and releases newly modeled PLs with a higher degree of unsaturation [11]. The *MBOAT7* mutations thus lead to the accumulation of intracellular free arachidonic acid, which is used as a substrate for the synthesis of inflammatory lipid mediators. In addition, the higher PI availability associated with weak *MBOAT7* enzymatic activity is used to synthesize diacylglycerols, which are the main precursors of triglycerides stored in lipid droplets [11].

In a cryo-EM structure model proposed by Wang and colleagues [12], the arachidonoyl-CoA substrate enters the enzyme tunnel from the cytoplasmic leaflet of the ER and lyso-PI enters the tunnel from a luminal leaflet with a side channel harboring its single acyl chain and positions the sn-2 hydroxyl group of the glycerol backbone near the His356 residue located in the catalytic domain [12].

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Human      MSPEEWTYLVVLLISIPIGFLFKKAGPGLKRWGAAAVGLGLTLFTCGPHTLHSLVTILGT 60
Rat        MTPEEWTYLMVLLISIPVGFLFKKAGPGLKRWGAAAVGLGLTLFTCGPHSLHSLITILGT 60
Mouse      MTPEEWTYLMVLLISIPVGFLFKKAGPGLKRWGAAAVGLGLTLFTCGPHSLHSLITILGT 60
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Human      WALIQAQPCSCHALALAWTFSYLLFFRALSLLGLPTPTPFTNAVQLLLTLKLVSLASEVQ 120
Rat        WALIQAQPCSCHALALAWTFSYLLFFRALSLLGLPTPTPFTNAVQLLLTLKLVSLASEVQ 120
Mouse      WALIQAQPCSCHALALAWTFSYLLFFRALSLLGLPTPTPFTNAVQLLLTLKLVSLASEVQ 120
           ************************************************************

Human      DLHLAQRKEMASGFSKGPTLGLLPDVPSLMETLSYSYCYVGIMTGPFFRYRTYLDWLEQP 180
Rat        DLHLAQRKEMASGFSKEPTLGLLPDVPSLMETLSYSYCYVGIMTGPFFRYRTYLDWLEQP 180
Mouse      DLHLAQRKEIASGFHKEPTLGLLPEVPSLMETLSYSYCYVGIMTGPFFRYRTYLDWLEQP 180
           *********:**** * *******:***********************************

Human      FPGAVPSLRPLLRRAWPAPLFGLLFLLSSHLFPLEAVREDAFYARPLPARLFYMIPVFFA 240
Rat        FPEAVPSLRPLLRRAWPAPLFGLLFLLSSHLFPLEAVREDAFYARPLPTRLFYMIPVFFA 240
Mouse      FPEAVPSLRPLLRRAWPAPLFGLLFLLSSHLFPLEAVREDAFYARPLPTRLFYMIPVFFA 240
           ** ********************************************:************

Human      FRMRFYVAWIAAECGCIAAGFGAYPVAAKARAGGGPTLQCPPPSSPEKAASLEYDYETIR 300
Rat        FRMRFYVAWIAAECGCIAAGFGAYPVAAKARAGGGPTLQCPPPSSPEIAASLEYDYEAIR 300
Mouse      FRMRFYVAWIAAECGCIAAGFGAYPVAAKARAGGGPTLQCPPPSSPEIAASLEYDYETIR 300
           ********************************************* *********:**

Human      NIDCYSTDFCVRVRDGMRYWNMTVQWWLAQYIYKSAPARSYVLRSAWTMLLSAYWHGLHP 360
Rat        NIDCYGTDFCVRVRDGMRYWNMTVQWWLAQYIYKSAPFRSYVLRSAWTMLLSAYWHGLHP 360
Mouse      NIDCYGTDFCVRVRDGMRYWNMTVQWWLAQYIYKSAPFRSYVLRSAWTMLLSAYWHGLHP 360
           *****.****************************** **********************

Human      GYYLSFLTIPLCLAAEGRLESALRGRLSPGGQKAWDWVHWFLKMRAYDYMCMGFVLLSLA 420
Rat        GYYLSFMTIPLCLAAEGYLESALRRHLSPGGQKAWDWFHWFLKMRAYDYMCMGFVLLSMG 420
Mouse      GYYLSFMTIPLCLAAEGYLESALRRHLSPGGQKAWDWVHWFLKMRAYDYMCMGFVLLSMA 420
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Human      DTLRYWASIYFCIHFLALAALGLGLALGGGSPSRRKAASQPTS-LAPEKLREE        472
Rat        DTLRYWASIYFWVHFLALACLGLGLALGGGSPSKRKTPSQATTSQAKEKLREE        473
Mouse      DTLRYWASIYFWVHFLALACLGLGLVLGGGSPSKRKTPSQATSSQAKEKLREE        473
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Figure 1. *MBOAT7*, a highly conserved O-acyltransferase. Sequence comparison of human, rat, and mouse *MBOAT7* proteins demonstrating the high degree of sequence similarity. Amino acid residues conserved in all three sequences are indicated by asterisks, while conserved amino acid residues with strongly similar properties are marked by colons, and amino acids with weakly similar properties are indicated by periods. The protein sequences were taken from protein sequences (human: NP_077274, rat: XP_038966897, mouse: NP_084210) deposited in the National Library of Medicine [13] and the alignment was performed using the EMBL-EBI Clustal Omega multiple sequence alignment tool [14]). Human *MBOAT7* is composed of 11 transmembrane helical segments that are connected by short intervening helices and loops. For more details about the structure of human *MBOAT7*, refer to [12]. The structure of human *MBOAT7* was generated with Jmol using the structure coordinates deposited in the RCSB Protein data base (access. no.: 8ERC).

3. Pathogenesis of NAFLD

NAFLD is defined by elevated hepatic lipid accumulation exceeding 5% of liver weight in the absence of heavy alcohol consumption. NAFLD entails a wide spectrum of condi-

tions ranging from uncomplicated steatosis to NASH progressing to fibrosis and cirrhosis with few cases further progressing to hepatocellular carcinoma (HCC) [15]. The pathogenesis of NAFLD is closely related to obesity, glucose intolerance, and dyslipidemia [15]. Unhealthy dietary habits, highly fructose-enriched diets, excessive calorie intake, and lack of exercise along with genetic predisposition are the major contributors to this pathological condition [16]. The biological associations between fatty liver and inherited risk factors and their interplay with environmental factors are primary goals in the study of NAFLD pathophysiology.

In addition, multiple studies have established a strong correlation between NAFLD and increased risk of cardiovascular complications [17]. A recent retrospective study showed that NAFLD determined early atherosclerosis and progression, independently of traditional cardiovascular risk factors [18]. Few longitudinal and retrospective studies have demonstrated interesting findings on NAFLD association with hypertension, chronic kidney disease, and cardiometabolic comorbidities [19]. It is certainly evident that dysregulation of lipid and glucose metabolism and activation of the prothrombotic system are some of the mechanisms involved in NAFLD leading to cardiometabolic complications.

A 'Multi-hit' hypothesis has been proposed in previous studies to understand NAFLD pathogenesis [20]. Insulin resistance is the first hit responsible for hepatic steatosis either due to impaired insulin receptor activity or derangement of insulin response through downstream signaling cascade. Insulin resistance favors lipid storage dismissal, increasing the efflux of free fatty acids from adipose tissue to the liver where they are stored as triglycerides [21]. In addition, fat deposition is exacerbated by hyperinsulinemia by inducing de novo lipogenesis from glucose through sterol regulatory element binding protein 1 (SREBP1). Mitochondria dysfunctions and changes in PUFAs are also responsible for steatosis onset [22].

Under normal conditions, there is a balance and dynamic equilibrium between fatty acid storage and expenditure, in which the liver is the key organ involved in regulatory systemic lipid metabolism via coordinating lipid uptake, synthesis, oxidation, and export. Peripheral lipolysis, de novo lipogenesis, dietary-derived chylomicrons, and intermediate density lipoprotein particles are the main free fatty acid sources contributing to hepatic lipid accumulation [23]. Insulin resistance decreases the utilization of triglycerides in peripheral tissues and increased lipolysis in adipose tissue, leading to increased influx of free fatty acids to the liver. In addition, increased hepatic influx of chylomicrons and very-low-density lipoprotein (VLDL) can be caused by decreased lysophosphatidylinositol activity. These mechanisms mainly contribute to excess triglycerides and TC accumulation in the liver, which in turn enhances the assembly of VLDL particles, further worsening dyslipidemia [23].

There is much evidence illustrating the association between NAFLD pathogenesis and inflammatory responses [24]. In NAFLD, hepatic immune cell populations exhibit an immunogenic phenotype composed of Kupffer cells, monocyte-derived macrophages, and dendritic cells, whose transcriptional alterations were elucidated by single-cell RNA-seq technology determining NAFLD progression [24]. In line with this, an interesting finding illustrated a NASH-associated macrophage subset, with TREM2 expression linked to NASH severity. In particular, a specialized circulating monocyte-derived TREM2 $CD9^{+}$ macrophage subpopulation that promotes liver fibrosis seems to play an essential role in NASH-associated fibrogenesis [25].

Recent clinical studies have shed light on NAFLD severity being associated with inflammatory markers such as TNF, IL-1, IL-6, and high-sensitivity C-reactive protein. It is suggested that NAFLD may induce systemic inflammation, insulin resistance, and oxidative stress via these pro-inflammatory molecules [26].

NAFLD is a perfect example of ectopic fat accumulation, meaning that lipid accumulation occurs in another site other than adipose tissue. This in turn is associated with increased secretion of hepatokines, increased gluconeogenesis, and inhibition of insulin signaling [27]. Hepatic lipid accumulation causes insulin resistance and chronic inflam-

mation, increasing the risk of fibrosis, cirrhosis, and HCC. Besides liver lipid metabolism, the other possibilities contributing to the pathogenesis of NAFLD include adipose tissue dysfunction/inflammation, dysbiosis of gut microbiota, and gut barrier function regulating several intrahepatic metabolic and inflammatory pathways (Figure 2) [28,29].

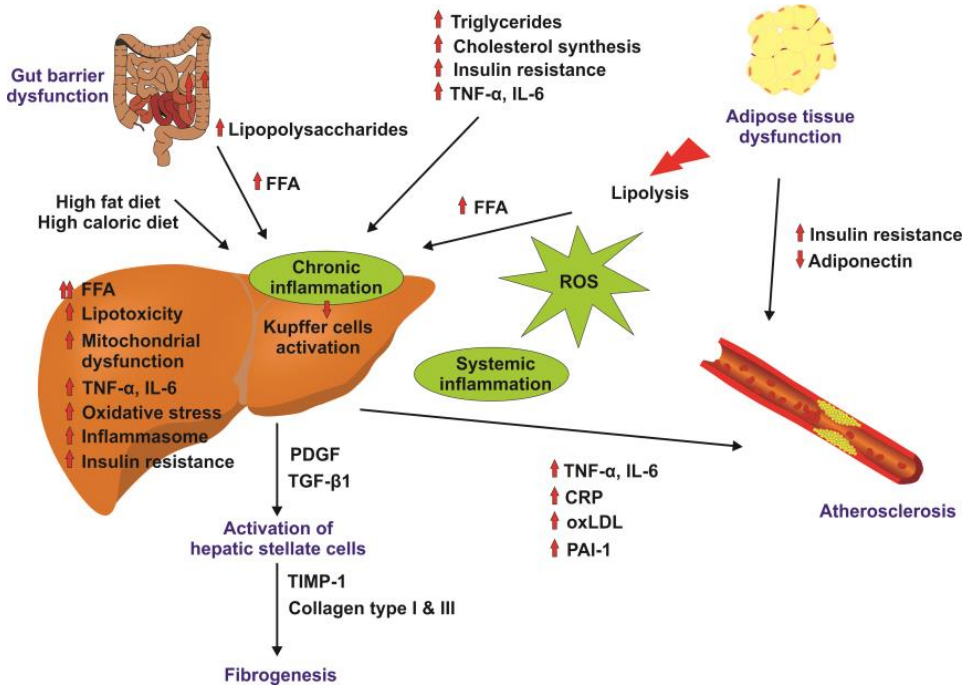

Figure 2. Factors driving pathogenesis of NAFLD and formation of atherosclerosis. Lipopolysaccharides that destroy the gut barrier function, high-caloric diets and elevated concentrations of free fatty acids (FFAs) lead to hepatic inflammation. This in turn leads to activation of hepatic stellate cells and production of cytokines (e.g., PDGF, TGF-β1) that drive fibrogenesis. Additionally, adipose tissue dysfunction and lipolysis leads to formation of insulin resistance and reactive oxygen species (ROS) that further drive the inflammatory process. Hepatic inflammation can result in systemic inflammation and factors (e.g., TNF-α, IL-6, CRP, oxLDL, PAI-1) that in concert with elevated concentrations of fat trigger atherosclerosis. Upward pointing arrows indicate increased concentrations/symptoms, while arrows pointing downwards lower concentrations/symptoms. This figure was redrawn in modified form from [29].

It is interesting to note that obesity is strongly associated with NAFLD but not an independent factor. Adipose tissue dysfunction is the key contributor to NAFLD in patients with lipodystrophy by increasing the hepatic/peripheral insulin resistance and promoting hepatic inflammation (Figure 3) [8,30].

In addition to these factors, the long chain fatty acids are esterified with glycerol-3-phosphate to mono-acylglycerols, diacylglycerols, and triacylglycerols in hepatocytes. The production of these intermediates is increased by lipid synthesis and lipid products like ceramides, which play crucial roles in causing resistance in the insulin signaling pathway, promoting hepatic inflammation and the progression of NAFLD [2,27].

Further, it is likely that the putative underlying mechanisms linking NAFLD, chronic kidney disease, and cardiovascular disease have their origin from inflamed visceral adipose tissue [4]. This in turn exacerbates atherogenic dyslipidemia, releasing a myriad of pro-inflammatory molecules, thrombogenic molecules, contributing to the pathophysiology of cardiovascular disease and chronic kidney disease. The co-existence of obesity further exerts additional effects, leading to functional derangements in the heart, kidneys, and vasculature [4].

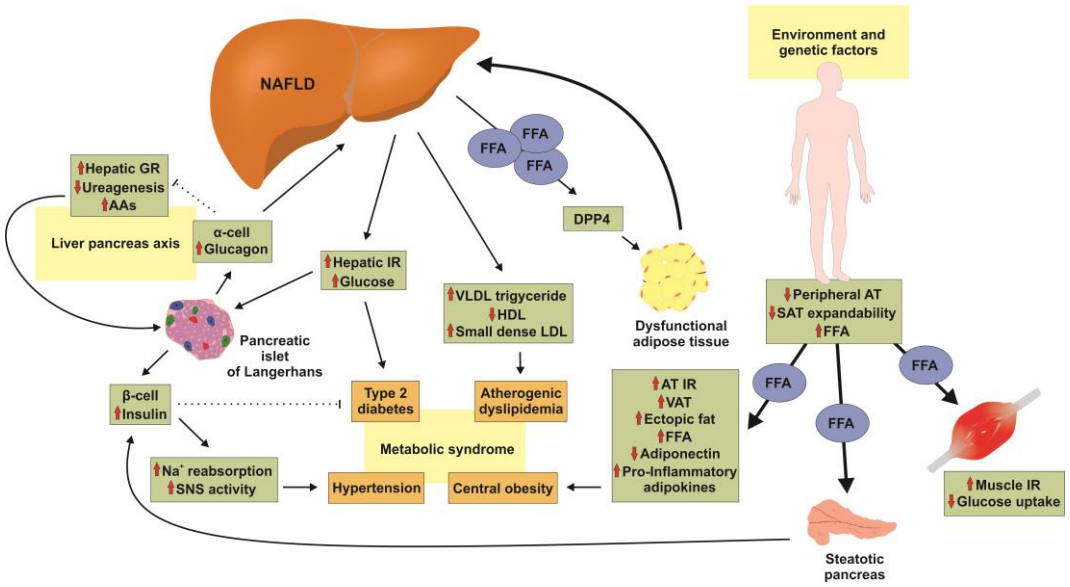

Figure 3. NAFLD as a result of obesity and metabolic syndrome. Increased free fatty acids (FFAs) result in dysfunction of peripheral adipose tissue (AT), expansion of subcutaneous adipose tissue SAT), central obesity, type 2 diabetes, insulin resistance (IR), and central obesity that form key features in the metabolic syndrome. These changes can be monitored by alterations in blood parameters (VLDL, triglyceride, small dense LDL, glucose). These changes are further associated with increased activity of sympathetic nervous system (SNS), glucagon increase, hepatic clearance of amino acids (AA), and increased ureagenesis. Epigenetic and genetic factors predispose to NAFLD pathogenesis. Upward pointing arrows indicate increased concentrations/symptoms, while arrows pointing downwards lower concentrations/symptoms. Solid arrows mark stimulatory effects, while dashed lines indicate inhibitory effects. For more information refer to the text. Abbreviations used are AA: arachidonic acid; GR: glucagon resistance; LDL: low-density lipoprotein(s). This figure was redrawn in modified form from [30].

4. Mechanistic Link between *MBOAT7* and NAFLD and Related In Vivo/In Vitro Studies

It is crucial to further understand the molecular mechanisms underlying the progression of liver disease from simple steatosis to more advanced fibrotic disease. *MBOAT7* association with NAFLD has emerged as a new lipid metabolic pathway as growing evidence suggests the pivotal role of *MBOAT7* as a driver of NAFLD development and progression. Few studies have provided vital clues into its broader role and mechanistic insights.

MBOAT7 preferentially esterifies lysophosphatidylinositol (LPI) lipids to arachidonoyl-CoA to form major phosphoinositol (PI) species (38:4) in the inner leaflet of cell membranes. Phospholipases, prominently phospholipase A2 (PLA2), cleave fatty acid from the sn2 position and *MBOAT7* selectively re-esterifies new PUFAs in that position, completing the remodeling cycle [31]. *MBOAT7* loss of function could alter cellular signal transduction, protein–lipid interactions, vesicular transport, and membrane fusion events, given the fact that *MBOAT7* generates the most abundant PI species (38:4) and key cellular phosphatidylinositol phosphates (PI 18:0/20:4) and PI ([18:0/20:4]-4,5P2). Another potential way by which *MBOAT7* loss of function could promote NASH is by abnormal accumulation of LPI substrates in liver, as evidenced by multiple mice studies. These LPI substrates serve as relevant lipid signals promoting pro-inflammatory and pro-fibrotic effects [31].

The liver-specific knockout of *MBOAT7* induces hepatic fat accumulation by increasing de novo lipogenesis driven by SREBP1, which is a key lipogenic transcription factor involved in fatty acid biosynthesis [22]. The non-canonical pathway, on the other hand, suggests that *MBOAT7* depletion causes a simultaneous increase in PI synthesis and PI degradation mediated by a protein with PLC activity resulting in diacylglycerol, a substrate of triglyceride synthesis [32].

Lee and coworkers unraveled the first evidence of *MBOAT7* enzymatic activity in *Caenorhabditis elegans* by RNA-interference-based genetic screening [33]. In *C. elegans*, eicosapentaenoic acid is the predominant PUFA, which is decreased by *MBOAT7* deletion. It also exhibited reduced PI species and PI3P-related events [33]. Similarly, it has been shown that obese people have low levels of *MBOAT7* in their livers and genetically modified obese mice with low *MBOAT7* levels developed more severe NAFLD [8]. Strikingly, excess fat accumulation was noticed in human liver cells with low levels of *MBOAT7*. New approaches to therapeutic strategies in treating NAFLD in patients with *MBOAT7* mutations can be developed with *MBOAT7* being a critical mediator of NAFLD.

Several studies revealed that the hepatic *MBOAT7* expression levels were suppressed in high-fat-diet mice and obese leptin-deficient mice and that *MBOAT7* levels in adipose tissue were negatively correlated with insulin sensitivity and impaired glucose tolerance (Figure 4) [21,34].

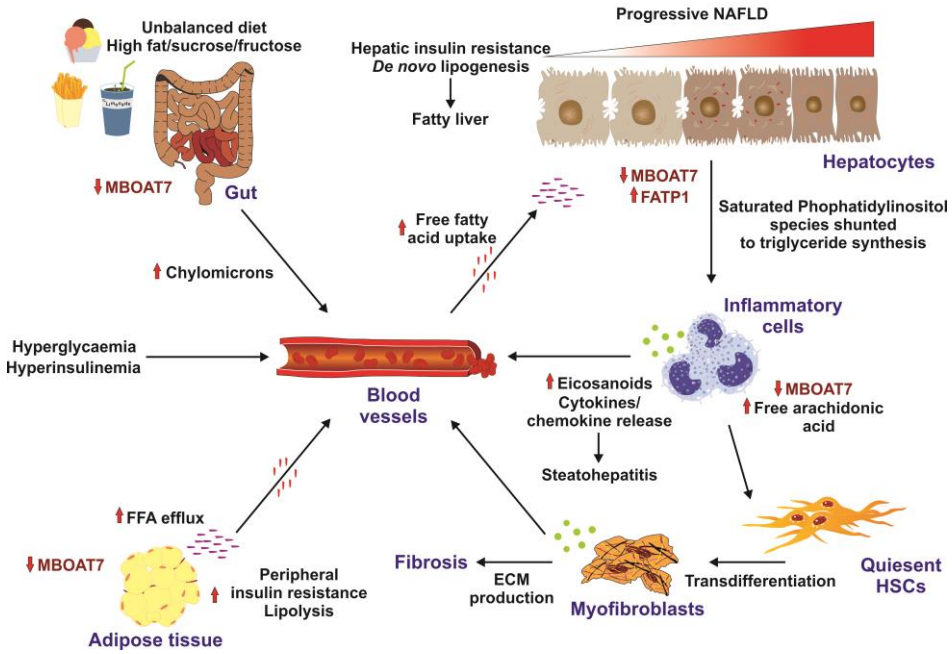

Figure 4. Factors promoting insulin resistance. High-caloric diets enriched in fat, sucrose, and fructose are risk factors for insulin resistance development. In the gut, the unbalanced diet results in downregulation of *MBOAT7* and formation of chylomicrons. Elevated concentrations of systemic free fatty acids are taken up by hepatocytes, resulting in increased de novo lipogenesis and fatty liver. This provokes the infiltration of the liver with inflammatory cells, which results in elevated concentrations of cytokines and chemokines, provoking steatohepatitis and transdifferentiation of quiescent hepatic stellate cells (HSCs) to extracellular-matrix-producing (ECM) myofibroblasts leading to hepatic fibrosis. Similarly, to the liver and the gut, *MBOAT7* is downregulated in the adipose, triggering peripheral insulin resistance. Solid arrows mark stimulatory effects. Small red lines indicate free fatty acids in the circulation, while small purple lines indicate tissue-bound free fatty acids. All these factors promote formation of hyperglycemia, hyperinsulinemia, and insulin resistance. This figure was redrawn in modified form from [34].

On a similar context, liver-specific deletion of *MBOAT7* increased liver fat content in chow-diet-fed mice under fasting-re-feeding conditions. This hepatic lipid accumulation is shown to be caused due to an increase in de novo lipogenesis driven by SREBP1, supported by normalization of hepatic fat content by liver-specific deletion of *MBOAT7* and SREBP cleavage-activating protein *Scap* [22].

In addition to the in vivo studies, Tanaka et al. investigated the impact of *MBOAT7* deletion on PI content and fat accumulation in cultured hepatocytes. This study suggested that depletion of *MBOAT7* in hepatocytes resulted in hepatic fat accumulation through

increased triglyceride synthesis [32]. Here, it was proposed that hepatic lipid accumulation is due to a novel non-canonical pathway supplying substrates from PI to triglycerides through a futile cycle [32].

A wealth of recent data show that *MBOAT7* overexpression in mice had beneficial effects on NASH pathology by significantly decreasing hepatic triglyceride levels and normalizing liver injury markers such as alanine aminotransferase and aspartate aminotransferase [35]. Longo et al. showed that *MBOAT7* rs641738 and TM6SF2 E167k alters lipid droplet accumulation, mitochondrial morphology, and metabolic reprogramming towards HCC in vitro [36]. Strikingly, Krawczyk et al. confirmed the association of *MBOAT7* with a more severe stage of fibrosis increasing plasma triglycerides, cholesterol, LDL, and glucose levels [37].

An interesting study by Raja et al. shed light on *MBOAT7* rs641738 associations with NAFLD based on ethnicity [38]. It was shown that *MBOAT7* is a strong contributor to the progression of NAFLD in the Caucasian and Chinese population, while it is not significant in Afro-American and Hispanic populations [39]. Yet another unique finding by Massey et al. showed diet-induced metabolic disturbances, hyperinsulinemia, and systemic insulin resistance in mice with adipocyte-specific disruption of the *MBOAT7* gene [21].

Furthermore, liver-specific knockdown of *MBOAT7* by antisense oligonucleotides revealed large alterations in liver lipid storage characterized by the accumulation of triglycerides, free cholesterol, and cholesterol esters in high-fat-diet-fed mice [8]. In addition, *MBOAT7* knockdown was associated with liver injury as indicated by elevated aspartate aminotransferase and alanine aminotransferase in these animals. Aligned with these alterations, knockdown of *MBOAT7* in high-fat-diet-fed mice resulted in alterations in LPI and PI lipids in a tissue-specific manner such as selective reduction in 38:3 and 38:4 species of circulating PI lipids and significant accumulation of 16:0 and 18:1 LPI species in the liver, inducing an imbalance of local lipid mediators that originate from PI metabolism.

A recent meta-analysis of 42 studies including more than 1 million participants showed that the *MBOAT7* variation is firmly associated with the severity of NAFLD in European adults [40]. Another interesting study suggests that *MBOAT7* is a negative regulator of TLR signaling and highlights that *MBOAT7* modulation can be beneficial for suppressing inflammation associated with the dysregulation of Toll-like receptor signaling such as metabolic-associated fatty liver diseases (MAFLD) [24].

5. Challenges and Recent Progress in Treating Complex NAFLD

NAFLD represents a 'silent epidemic' as it is often asymptomatic in nature with increased prevalence among adults with obesity, type 2 diabetes, insulin resistance, and metabolic syndrome [41]. It represents a growing public health challenge owing to lack of approved therapies at present [42]. Individuals with NAFLD present at least one feature of metabolic syndrome, making it an even more challenging multi-systemic disease. In addition, the complexity is increased by the fact that many patients remain undiagnosed in early phases of the disease [43]. Variability in NAFLD-related risk factors, substantial mortality, and morbidity are a few other challenges in the management of this disease [43]. Therefore, it is important to adopt a holistic approach in managing this diversified condition.

At present, NAFLD is treated with lifestyle modifications such as weight loss and dietary changes as there is no approved therapy yet for this multi-factorial disease [42]. Multiple drug strategies are being developed and tested to treat advanced NAFLD and target inflammatory, fibrotic, and metabolic pathways. More recently, a structure and model were reported for the catalytic mechanism of human *MBOAT7*, which reveals a twisted tunnel from the cytosol and luminal side providing access for arachidonoyl-CoA and lyso-PI. This structure might be important in the identification of small molecule inhibitors for targeted drug therapy in treating NAFLD [12].

There is one other study, which elucidated that increased expression of *MBOAT7* is co-related with detrimental outcomes in HCC, emphasizing the role of *MBOAT7* inhibitors as useful therapeutic targets in treating HCC [44].

A profound study by Thangapandi et al. provided combined mice and human datasets and demonstrated that targeting PI signaling might be a potential therapeutic option for treating NAFLD and fibrosis. Their study unfolded a novel finding that *MBOAT7* deficiency in mice and humans points to an inflammation-independent pathway of liver fibrosis mediated by lipid signaling, opening new avenues for potential targets for NAFLD [45]. It was also shown via lipidomics that in addition to PI and LPI, phosphoglycerol, lysophophatidylglycerol, and phosphatidic acids were increased in *MBOAT7*-deficient livers.

For patients with biopsy-proven NASH, vitamin E and pioglitazone supplements are recommended, although there are concerns regarding side effects [46]. As previously mentioned, intestinal microbiota play an important role in NAFLD pathogenesis. Thus, probiotics, antibiotics, and prebiotics might play a therapeutic role via modulating gut microbiota [47].

Genetic screening for polymorphisms to identify the individuals at high risk for NAFLD will help in deeper understanding of specific therapeutic strategies. Modulators of bile acid signaling medications to improve insulin sensitivity are being tested in patients with NASH as a possible therapeutic approach [48].

Currently, there are several innovative pipeline drugs that are tested in clinical trials for the treatment of NAFLD/NASH (Figure 5).

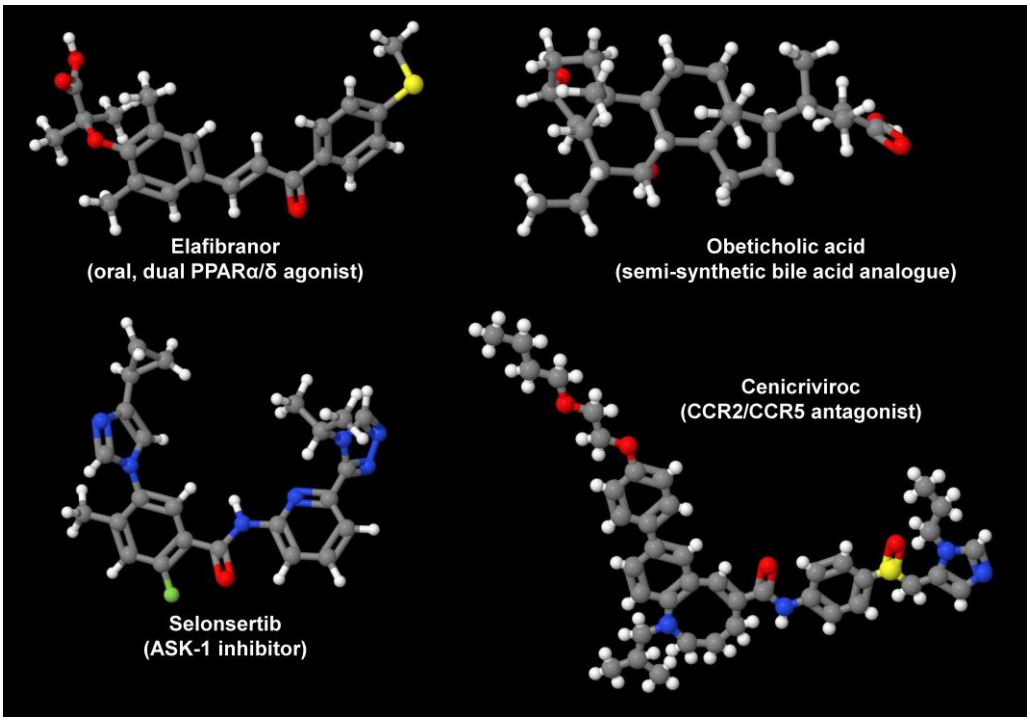

Figure 5. Drug candidates for treatment of NAFLD/NASH. Depicted are the oral, dual PPARα/δ agonist Elafibranor, the semi-synthetic bile acid analogue obeticholic acid, the ASK-1 inhibitor Selonsertib, and the CCR2/CCR5 antagonist Cenicriviroc. These are only some drugs that are tested in ongoing studies. In addition, there are several TLR4 antagonists and macrolide antibiotics that are tested in ongoing trials. Their variable mode of action demonstrates that there are many independent options to target the pathogenesis of NAFLD/NASH. All structures depicted were generated with the open-source molecule viewer Jmol, version 14.2.15_2015.07.09 [49] using information depicted in PubChem [50] for Elafibranor (PubChem CID: 9864881), obeticholic acid (PubChem CID: 447715), Selonsertib (PubChem CID: 71245288), and Cenicriviroc (PubChem CID: 11285792), respectively.

Elafibranor, which acts as a dual-peroxisome proliferation-activated receptor (PPAR) α/δ agonist, improves glucose metabolism and insulin sensitivity and decreases inflammation. In a phase 2b randomized control study (NCT0164849, GOLDEN-505), the effects of Elafibranor (120.80 mg/day) were studied for 52 weeks. NASH was resolved in 19% of

patients compared to 9% in the placebo group. In addition, a phase 3 RCT (NCT02704403, RESOLVE-IT) is being evaluated on the effects of Elafibranor (120 mg/day for 72 weeks). Additional novel PPAR agonists such as Saroglitazar and Lanifibranor are also being tested [51].

Obeticholic acid, a semi-synthetic bile activator of Farnesoid X receptors, regulates lipid/glucose homeostasis, promotes insulin sensitivity, and modulates liver fibrosis. In a phase 2b RCT (NCT01264598, FLINT), obeticholic acid (25 mg/day) was tested for 72 weeks in patients with a NAFLD score greater than 4. Remarkably, 45% of the patients showed histological improvement compared with 21% in the placebo group [52]. This trial progressed to a phase 3 RCT (NCT02548351 REGENERATE), which is currently recruiting patients with biopsy-proven NASH to assess obeticholic acid's effects and to evaluate the long-term effects over a 7-year period [53].

The involvement of inflammatory cells and cascade of inflammatory events is well known in hepatocyte injury. Cenicriviroc (CVC) functions as a dual antagonist of CCR2 and CCR5 and demonstrated decreased fibrosis in preclinical models. A phase 2 RCT (NCT02217475, CENTAUR) with CVC 150 mg/day was evaluated in 289 patients with NASH, fibrosis, and diabetes mellitus. Although there was no significant improvement in NASH after 12 months, liver fibrosis improved in 20% patients as compared to 10% in the placebo group. A phase 3 RCT (NCT03028740 AURORA) was initiated to evaluate the effects of 150 mg/day CVC with long-term follow-up over a 5-year period [54].

TNF-α signaling plays an important role in hepatocyte injury and apoptosis, which in turn activates the apoptosis signal-regulating kinase 1 (ASK1) leading to hepatic inflammation, fibrosis, and hepatocyte apoptosis. Selonsertib, a selective inhibitor of ASK1, was tested for its effects on patients with NASH and fibrosis in a phase 2 RCT (NCT02466516). The patients showed fibrosis improvement after 24 weeks of treatment. Two phase 3 trials (NCT03053050 STELLAR3, NCT03053063 STELLAR 4) are being conducted currently to evaluate the effects of Selonsertib at week 48 and further monitor at 240 weeks [55].

In principle, there are also ways to directly target *MBOAT7* gene expression or activity. Since the suppression of *MBOAT7* was shown to drive hepatic fat accumulation and NAFLD development [8,56], the overexpression of *MBOAT7* or approaches leading to increased endogenous expression of *MBOAT7* should have beneficial effects on the outcome of NAFLD. Therapeutic gene therapy has been used in a plethora of diseases so far [57]. In particular, engineered hepatotropic adeno-associated viruses, retroviral vectors, or lentiviral delivery systems expressing *MBOAT7* under transcriptional control of liver-specific promoters could be used to increase the overall concentration of *MBOAT7* in the liver. Similarly, the transfer of nanoparticles, liposomes, polymers, virus-like particles, erythrocyte ghosts, and exosomes that are loaded with *MBOAT7* expression constructs, or special in vivo or ex vivo gene transfer techniques might be scalable alternatives to increase MBOAT quantities [57].

Similarly, strategies that enhance the translation and stability of endogenous or exogenous mRNA that were already used in other disease scenarios could be applied [58]. Finally, in the long term, there will be gene replacement techniques available (e.g., CRISPR/Cas9) that will allow for the replacement of *MBOAT7* mutations that are associated with increased accumulation of intracellular free fatty acids and hepatic steatosis. Nevertheless, although gene therapy is a promising therapeutic strategy that made remarkable advancements during the last decade, there are still many hurdles in the use of this promising therapy [59].

Nevertheless, a proof-of-concept study has recently shown that the overexpression of *MBOAT7* in mice fed either a choline-deficient high-fat diet or a Gubra Amylin NASH diet and subsequent infected with an adeno-associated virus expressing *MBOAT7* failed to improve in terms of NASH pathology [35]. However, in the mentioned study, the authors demonstrated that *MBOAT7* overexpression slightly improved liver weights, triglycerides, and plasma alanine and aspartate transaminases [35]. It is possible that *MBOAT7* needs additional factors to be therapeutically effective in NAFLD/NASH. This again highlights the complexity of the NAFLD/NASH pathogenesis that is driven by many genetic and

epigenetic factors and pinpoints the fact that further studies are urgently needed to identify proper targeted therapies for NALFD/NASH.

6. Future Directions in Management of NAFLD

Collectively, NAFLD is a complex disease associated with increased adiposity, diabetes mellitus, and insulin resistance. Patients with mild NAFLD, which is relatively benign, are usually managed with lifestyle modifications through diet and exercise, whereas patients with NASH and fibrosis are usually managed with different classes of medications along with lifestyle modifications. The treatment of the final stages of disease progression, which are NASH-induced cirrhosis and HCC, is focused on preventing and treating the complications associated with it and liver transplant as a last resort [60]. Although lifestyle interventions are beneficial, the effects are short-lived. Additional research is needed to identify the most effective and customized treatment strategies for treating NASH in different patient populations.

7. Conclusions

NAFLD imposes a huge health burden globally due to its risk of progression to cirrhosis, fibrosis, and HCC and lack of well-defined approved therapies. NAFLD is the most common chronic liver disorder affecting more than one-third of the population worldwide and its pathogenesis is closely related to insulin resistance, dyslipidemia, obesity, and adipose tissue dysfunction. The pathogenesis of NAFLD is based on a 'Multihit' hypothesis with the main hit being hepatic triglyceride accumulation and susceptibility to liver injury by the increased influx of free fatty acids in insulin resistance and obesity. This is mediated by inflammatory cytokines, mitochondrial dysfunction, and oxidative stress leading to steatohepatitis and fibrosis. In the last few years, some robust studies elucidated the associations between genetic polymorphisms (e.g., in *PNPLA3*, *TM6SF2*, *GCKR*, *HSD17B13*, *PSD3*, *APOE*, and *MBOAT7*) and NAFLD. Single-nucleotide polymorphisms in *MBOAT7* are broadly associated with increased risk of initiation and progression of NAFLD to NASH and fibrosis and in a few cases to HCC. Multiple in vitro and in vivo studies have unraveled that *MBOAT7* deficiency in mice and humans alters the hepatic PL composition and LPI to promote hyperinsulinemia and hepatic insulin resistance. *MBOAT7* catalyzes the desaturation of the second acyl chain of PLs and transfers PUFAs, in the form of acyl-CoA to lyso-PLs, using arachidonic acid as a substrate, regulating the amount of free arachidonic acid, which is a potent trigger for hepatic inflammation and fibrosis and a precursor of multiple pro-inflammatory mediators such as eicosanoids. The treatment of this multi-spectrum disease is complex and challenging as there are plethora of factors involved in its pathogenesis and progression. Currently, there is no approved standard therapy for treating NAFLD. Multiple drugs are in phase 2 and phase 3 trials with the goal of developing potent and customized treatment strategies. An improved knowledge of pathophysiological links between NAFLD and *MBOAT7* will certainly help decrease the global burden of this complicated, wide-spectrum disease by opening new horizons in treatment strategies.

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References

1. Dowman, J.K.; Tomlinson, J.W.; Newsome, P.N. Pathogenesis of non-alcoholic fatty liver disease. *Qjm* **2010**, *103*, 71–83. [CrossRef]
2. Anstee, Q.M.; Targher, G.; Day, C.P. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat. Rev. Gastroenterol. Hepatol.* **2013**, *10*, 330–344. [CrossRef]
3. Shaker, M.; Tabbaa, A.; Albeldawi, M.; Alkhouri, N. Liver transplantation for nonalcoholic fatty liver disease: New challenges and new opportunities. *World J. Gastroenterol.* **2014**, *20*, 5320–5330. [CrossRef]
4. Armstrong, M.J.; Adams, L.A.; Canbay, A.; Syn, W.K. Extrahepatic complications of nonalcoholic fatty liver disease. *Hepatology* **2014**, *59*, 1174–1197. [CrossRef]
5. Nagral, A.; Bangar, M.; Menezes, S.; Bhatia, S.; Butt, N.; Ghosh, J.; Manchanayake, J.H.; Mahtab, M.A.; Singh, S.P. Gender differences in nonalcoholic fatty liver disease. *Euroasian J. Hepatogastroenterol.* **2022**, *12*, S19–S25. [CrossRef]
6. Caddeo, A.; Spagnuolo, R.; Maurotti, S. *MBOAT7* in liver and extrahepatic diseases. *Liver Int.* **2023**, *43*, 2351–2364. [CrossRef]
7. Mancina, R.M.; Dongiovanni, P.; Petta, S.; Pingitore, P.; Meroni, M.; Rametta, R.; Borén, J.; Montalcini, T.; Pujia, A.; Wiklund, O.; et al. The *MBOAT7*-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. *Gastroenterology* **2016**, *150*, 1219–1230.e1216. [CrossRef]
8. Helsley, R.N.; Varadharajan, V.; Brown, A.L.; Gromovsky, A.D.; Schugar, R.C.; Ramachandiran, I.; Fung, K.; Kabbany, M.N.; Banerjee, R.; Neumann, C.K.; et al. Obesity-linked suppression of membrane-bound O-acyltransferase 7 (*MBOAT7*) drives non-alcoholic fatty liver disease. *eLife* **2019**, *8*, e49882. [CrossRef]
9. Tavaglione, F.; Kono, N.; Romeo, S. Understanding the underlying molecular pathways by which *MBOAT7*/Lpiat1 depletion induces hepatic steatosis. *J. Lipid Res.* **2021**, *62*, 100047. [CrossRef]
10. Caddeo, A.; Jamialahmadi, O.; Solinas, G.; Pujia, A.; Mancina, R.M.; Pingitore, P.; Romeo, S. *MBOAT7* is anchored to endomembranes by six transmembrane domains. *J. Struct. Biol.* **2019**, *206*, 349–360. [CrossRef]
11. Lee, H.-C.; Inoue, T.; Sasaki, J.; Kubo, T.; Matsuda, S.; Nakasaki, Y.; Hattori, M.; Tanaka, F.; Udagawa, O.; Kono, N.; et al. LPIAT1 regulates arachidonic acid content in phosphatidylinositol and is required for cortical lamination in mice. *Mol. Biol. Cell* **2012**, *23*, 4689–4700. [CrossRef]
12. Wang, K.; Lee, C.-W.; Sui, X.; Kim, S.; Wang, S.; Higgs, A.B.; Baublis, A.J.; Voth, G.A.; Liao, M.; Walther, T.C.; et al. The structure of phosphatidylinositol remodeling *MBOAT7* reveals its catalytic mechanism and enables inhibitor identification. *Nat. Commun.* **2023**, *14*, 3533. [CrossRef]
13. National Library of Medicine. Available online: https://www.ncbi.nlm.nih.gov/protein (accessed on 9 November 2023).
14. Clustal Omega. Available online: https://www.ebi.ac.uk/Tools/msa/clustalo/ (accessed on 9 November 2023).
15. Byrne, C.D.; Targher, G. NAFLD: A multisystem disease. *J. Hepatol.* **2015**, *62*, S47–S64. [CrossRef]
16. Byrne, C.D. Ectopic fat, insulin resistance and non-alcoholic fatty liver disease. *Proc. Nutr. Soc.* **2013**, *72*, 412–419. [CrossRef]
17. Cai, J.; Zhang, X.-J.; Ji, Y.-X.; Zhang, P.; She, Z.-G.; Li, H. Nonalcoholic fatty liver disease pandemic fuels the upsurge in cardiovascular diseases. *Circ. Res.* **2020**, *126*, 679–704. [CrossRef]
18. Pais, R.; Redheuil, A.; Cluzel, P.; Ratziu, V.; Giral, P. Relationship among fatty liver, specific and multiple-site atherosclerosis, and 10-year Framingham Score. *Hepatology* **2019**, *69*, 1453–1463. [CrossRef]
19. Cheung, A.; Ahmed, A. Nonalcoholic fatty liver disease and chronic kidney disease: A review of links and risks. *Clin. Exp. Gastroenterol.* **2021**, *14*, 457–465. [CrossRef]
20. Buzzetti, E.; Pinzani, M.; Tsochatzis, E.A. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* **2016**, *65*, 1038–1048. [CrossRef]
21. Massey, W.J.; Varadharajan, V.; Banerjee, R.; Brown, A.L.; Horak, A.J.; Hohe, R.C.; Jung, B.M.; Qiu, Y.; Chan, E.R.; Pan, C.; et al. *MBOAT7*-driven lysophosphatidylinositol acylation in adipocytes contributes to systemic glucose homeostasis. *J. Lipid Res.* **2023**, *64*, 100349. [CrossRef]
22. Xia, M.; Chandrasekaran, P.; Rong, S.; Fu, X.; Mitsche, M.A. Hepatic deletion of *MBOAT7* (LPIAT1) causes activation of SREBP-1c and fatty liver. *J. Lipid Res.* **2021**, *62*, 100031. [CrossRef]
23. Ipsen, D.H.; Lykkesfeldt, J.; Tveden-Nyborg, P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell. Mol. Life Sci.* **2018**, *75*, 3313–3327. [CrossRef]
24. Alharthi, J.; Bayoumi, A.; Thabet, K.; Pan, Z.; Gloss, B.S.; Latchoumanin, O.; Lundberg, M.; Twine, N.A.; McLeod, D.; Alenizi, S.; et al. A metabolic associated fatty liver disease risk variant in *MBOAT7* regulates toll like receptor induced outcomes. *Nat. Commun.* **2022**, *13*, 7430. [CrossRef]
25. Zahr, T.; Sun, K.; Qiang, L. The polarizable and reprogrammable identity of Kupffer cells in nonalcoholic steatohepatitis. *Med. Rev.* **2022**, *2*, 324–327. [CrossRef]

26. Duan, Y.; Pan, X.; Luo, J.; Xiao, X.; Li, J.; Bestman, P.L.; Luo, M. Association of inflammatory cytokines with non-alcoholic fatty liver disease. *Front. Immunol.* **2022**, *13*, 880298. [CrossRef]
27. Zhu, B.; Chan, S.L.; Li, J.; Li, K.; Wu, H.; Cui, K.; Chen, H. Non-alcoholic steatohepatitis pathogenesis, diagnosis, and treatment. *Front. Cardiovasc. Med.* **2021**, *8*, 742382. [CrossRef]
28. Mehal, W.Z. The Gordian Knot of dysbiosis, obesity and NAFLD. *Nat. Rev. Gastroenterol. Hepatol.* **2013**, *10*, 637–644. [CrossRef]
29. Galatou, E.; Mourelatou, E.; Hatziantoniou, S.; Vizirianakis, I.S. Nonalcoholic steatohepatitis (NASH) and atherosclerosis: Explaining their pathophysiology, association and the role of incretin-based drugs. *Antioxidants* **2022**, *11*, 1060. [CrossRef]
30. Godoy-Matos, A.F.; Silva Júnior, W.S.; Valerio, C.M. NAFLD as a continuum: From obesity to metabolic syndrome and diabetes. *Diabetol. Metab. Syndr.* **2020**, *12*, 60. [CrossRef]
31. Varadharajan, V.; Massey, W.J.; Brown, J.M. Membrane-bound O-acyltransferase 7 (*MBOAT7*)-driven phosphatidylinositol remodeling in advanced liver disease. *J. Lipid Res.* **2022**, *63*, 100234. [CrossRef]
32. Yuki, T.; Yuta, S.; Andrea, C.; Takuya, K.; Yanli, M.; Tetsuya, K.; Naoto, K.; Toshimasa, Y.; Rosellina Margherita, M.; Guido, B.; et al. LPIAT1/*MBOAT7* depletion increases triglyceride synthesis fueled by high phosphatidylinositol turnover. *Gut* **2021**, *70*, 180. [CrossRef]
33. Lee, H.-C.; Inoue, T.; Imae, R.; Kono, N.; Shirae, S.; Matsuda, S.; Gengyo-Ando, K.; Mitani, S.; Arai, H. *Caenorhabditis elegans* mboa-7, a member of the MBOAT family, is required for selective incorporation of polyunsaturated fatty acids into phosphatidylinositol. *Mol. Biol. Cell* **2007**, *19*, 1174–1184. [CrossRef]
34. Meroni, M.; Longo, M.; Fracanzani, A.L.; Dongiovanni, P. *MBOAT7* down-regulation by genetic and environmental factors predisposes to MAFLD. *eBioMedicine* **2020**, *57*, 102866. [CrossRef]
35. Sharpe, M.C.; Pyles, K.D.; Hallcox, T.; Kamm, D.R.; Piechowski, M.; Fisk, B.; Albert, C.J.; Carpenter, D.H.; Ulmasov, B.; Ford, D.A.; et al. Enhancing hepatic *MBOAT7* expression in mice with nonalcoholic steatohepatitis. *Gastro Hep Adv.* **2023**, *2*, 558–572. [CrossRef]
36. Longo, M.; Meroni, M.; Paolini, E.; Erconi, V.; Carli, F.; Fortunato, F.; Ronchi, D.; Piciotti, R.; Sabatini, S.; Macchi, C.; et al. TM6SF2/PNPLA3/*MBOAT7* Loss-of-function genetic variants impact on NAFLD development and progression both in patients and in in vitro models. *Cell. Mol. Gastroenterol. Hepatol.* **2022**, *13*, 759–788. [CrossRef]
37. Krawczyk, M.; Rau, M.; Schattenberg, J.M.; Bantel, H.; Pathil, A.; Demir, M.; Kluwe, J.; Boettler, T.; Lammert, F.; Geier, A. Combined effects of the PNPLA3 rs738409, TM6SF2 rs58542926, and *MBOAT7* rs641738 variants on NAFLD severity: A multicenter biopsy-based study. *J. Lipid Res.* **2017**, *58*, 247–255. [CrossRef]
38. Raja, A.M.; Ciociola, E.; Ahmad, I.N.; Dar, F.S.; Naqvi, S.M.S.; Moaeen-Ud-Din, M.; Kaukab Raja, G.; Romeo, S.; Mancina, R.M. Genetic susceptibility to chronic liver disease in individuals from Pakistan. *Int. J. Mol. Sci.* **2020**, *21*, 3558. [CrossRef]
39. Sookoian, S.; Flichman, D.; Garaycoechea, M.E.; Gazzi, C.; Martino, J.S.; Castaño, G.O.; Pirola, C.J. Lack of evidence supporting a role of TMC4-rs641738 missense variant—*MBOAT7*- intergenic downstream variant—In the susceptibility to nonalcoholic fatty liver disease. *Sci. Rep.* **2018**, *8*, 5097. [CrossRef]
40. Teo, K.; Abeysekera, K.W.M.; Adams, L.; Aigner, E.; Anstee, Q.M.; Banales, J.M.; Banerjee, R.; Basu, P.; Berg, T.; Bhatnagar, P.; et al. rs641738C>T near *MBOAT7* is associated with liver fat, ALT and fibrosis in NAFLD: A meta-analysis. *J. Hepatol.* **2021**, *74*, 20–30. [CrossRef]
41. Sivell, C. Nonalcoholic fatty liver disease: A silent epidemic. *Gastroenterol. Nurs.* **2019**, *42*, 428–434. [CrossRef]
42. Meroni, M.; Longo, M.; Rustichelli, A.; Dongiovanni, P. Nutrition and genetics in NAFLD: The perfect binomium. *Int. J. Mol. Sci.* **2020**, *21*, 2986. [CrossRef]
43. Arab, J.P.; Díaz, L.A.; Dirchwolf, M.; Mark, H.E.; Lazarus, J.V.; Vaughan, E.; Méndez-Sánchez, N.; Oliveira, C.P.; Gadano, A.; Arrese, M. NAFLD: Challenges and opportunities to address the public health problem in Latin America. *Ann. Hepatol.* **2021**, *24*, 100359. [CrossRef]
44. Donati, B.; Dongiovanni, P.; Romeo, S.; Meroni, M.; McCain, M.; Miele, L.; Petta, S.; Maier, S.; Rosso, C.; De Luca, L.; et al. *MBOAT7* rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. *Sci. Rep.* **2017**, *7*, 4492. [CrossRef]
45. Thangapandi, V.R.; Knittelfelder, O.; Brosch, M.; Patsenker, E.; Vvedenskaya, O.; Buch, S.; Hinz, S.; Hendricks, A.; Nati, M.; Herrmann, A.; et al. Loss of hepatic *MBOAT7* leads to liver fibrosis. *Gut* **2021**, *70*, 940–950. [CrossRef]
46. Bril, F.; Biernacki, D.M.; Kalavalapalli, S.; Lomonaco, R.; Subbarayan, S.K.; Lai, J.; Tio, F.; Suman, A.; Orsak, B.K.; Hecht, J.; et al. Role of vitamin E for nonalcoholic steatohepatitis in patients with type 2 diabetes: A randomized controlled trial. *Diabetes Care* **2019**, *42*, 1481–1488. [CrossRef]
47. Iacono, A.; Raso, G.M.; Canani, R.B.; Calignano, A.; Meli, R. Probiotics as an emerging therapeutic strategy to treat NAFLD: Focus on molecular and biochemical mechanisms. *J. Nutr. Biochem.* **2011**, *22*, 699–711. [CrossRef]
48. Fiorucci, S.; Biagioli, M.; Sepe, V.; Zampella, A.; Distrutti, E. Bile acid modulators for the treatment of nonalcoholic steatohepatitis (NASH). *Expert Opin. Investig. Drugs* **2020**, *29*, 623–632. [CrossRef]
49. Jmol: An Open-Source Java Viewer for Chemical Structures in 3D with Features for Chemicals, Crystals, Materials and Biomolecules. Available online: https://jmol.sourceforge.net/ (accessed on 9 November 2023).
50. PubChem. National Library of Medicine. Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 9 November 2023).
51. Lange, N.F.; Graf, V.; Caussy, C.; Dufour, J.F. PPAR-targeted therapies in the treatment of non-alcoholic fatty liver disease in diabetic patients. *Int. J. Mol. Sci.* **2022**, *23*, 4305. [CrossRef]

52. Neuschwander-Tetri, B.A.; Loomba, R.; Sanyal, A.J.; Lavine, J.E.; Van Natta, M.L.; Abdelmalek, M.F.; Chalasani, N.; Dasarathy, S.; Diehl, A.M.; Hameed, B.; et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. *Lancet* **2015**, *385*, 956–965. [CrossRef]
53. Carr, R.M.; Reid, A.E. FXR agonists as therapeutic agents for non-alcoholic fatty liver disease. *Curr. Atheroscler. Rep.* **2015**, *17*, 500. [CrossRef]
54. Lefebvre, E.; Moyle, G.; Reshef, R.; Richman, L.P.; Thompson, M.; Hong, F.; Chou, H.L.; Hashiguchi, T.; Plato, C.; Poulin, D.; et al. Antifibrotic effects of the dual CCR2/CCR5 antagonist Cenicriviroc in animal models of liver and kidney fibrosis. *PLoS ONE* **2016**, *11*, e0158156. [CrossRef]
55. Harrison, S.A.; Wong, V.W.; Okanoue, T.; Bzowej, N.; Vuppalanchi, R.; Younes, Z.; Kohli, A.; Sarin, S.; Caldwell, S.H.; Alkhouri, N.; et al. Selonsertib for patients with bridging fibrosis or compensated cirrhosis due to NASH: Results from randomized phase III STELLAR trials. *J. Hepatol.* **2020**, *73*, 26–39. [CrossRef] [PubMed]
56. Meroni, M.; Dongiovanni, P.; Longo, M.; Carli, F.; Baselli, G.; Rametta, R.; Pelusi, S.; Badiali, S.; Maggioni, M.; Gaggini, M.; et al. *MBOAT7* down-regulation by hyper-insulinemia induces fat accumulation in hepatocytes. *eBioMedicine* **2020**, *52*, 102658. [CrossRef] [PubMed]
57. Sayed, N.; Allawadhi, P.; Khurana, A.; Singh, V.; Navik, U.; Pasumarthi, S.K.; Khurana, I.; Banothu, A.K.; Weiskirchen, R.; Bharani, K.K. Gene therapy: Comprehensive overview and therapeutic applications. *Life Sci.* **2022**, *294*, 120375. [CrossRef] [PubMed]
58. Kairuz, D.; Singh, P.; Smith, T.; Arbuthnot, P.; Ely, A.; Bloom, K. Synthetic mRNA gene therapies and hepatotropic non-viral vectors for the treatment of chronic HBV infections. In *Messenger RNA Therapeutics, RNA Technologies*; Jurga, S., Barciszewski, J., Eds.; Springer: Cham, Switzerland, 2022; Volume 13. [CrossRef]
59. Khurana, A.; Sayed, N.; Singh, V.; Khurana, I.; Allawadhi, P.; Rawat, P.S.; Navik, U.; Pasumarthi, S.K.; Bharani, K.K.; Weiskirchen, R. A comprehensive overview of CRISPR/Cas 9 technology and application thereof in drug discovery. *J. Cell. Biochem.* **2022**, *123*, 1674–1698. [CrossRef]
60. Burra, P.; Becchetti, C.; Germani, G. NAFLD and liver transplantation: Disease burden, current management and future challenges. *JHEP Rep.* **2020**, *2*, 100192. [CrossRef]