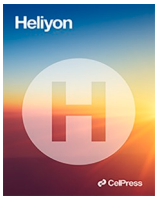
Heliyon 10 (2024) e30387

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

From NAFLD to NASH: Understanding the spectrum of non-alcoholic liver diseases and their consequences

Ahmed M. Samy [a], Mohamed A. Kandeil [b], Dina Sabry [c,d], A.A. Abdel-Ghany [a,e], Mohamed O. Mahmoud [f,*]

[a] Department of Biochemistry, Faculty of Pharmacy, Nahda University, Beni-Suef 62513, Egypt
[b] Department of Biochemistry, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt
[c] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo 11562, Egypt
[d] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Badr University in Cairo, Cairo 11829, Egypt
[e] Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Assuit Branch, Egypt
[f] Department of Biochemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt

ARTICLE INFO

Keywords:
NAFLD
NASH
Cirrhosis
Metabolic disorders
Gut microbiota

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) has become one of the most frequent chronic liver diseases worldwide in recent decades. Metabolic diseases like excessive blood glucose, central obesity, dyslipidemia, hypertension, and liver function abnormalities cause NAFLD. NAFLD significantly increases the likelihood of liver cancer, heart disease, and mortality, making it a leading cause of liver transplants. Non-alcoholic steatohepatitis (NASH) is a more advanced form of the disease that causes scarring and inflammation of the liver over time and can ultimately result in cirrhosis and hepatocellular carcinoma. In this review, we briefly discuss NAFLD's pathogenic mechanisms, their progression into NASH and afterward to NASH-related cirrhosis. It also covers disease epidemiology, metabolic mechanisms, glucose and lipid metabolism in the liver, macrophage dysfunction, bile acid toxicity, and liver stellate cell stimulation. Additionally, we consider the contribution of intestinal microbiota, genetics, epigenetics, and ecological factors to fibrosis progression and hepatocellular carcinoma risk in NAFLD and NASH patients.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver illness worldwide. NAFLD is a type of metabolic syndrome that manifests in the liver and is often accompanied by other metabolic risk factors such as dyslipidemia, diabetes, high blood pressure, and obesity. NAFLD prevalence is rising alongside global trends in obesity and type II diabetes [1].

In this review, we sought out information on the prevalence and development of NAFLD and its subsequent stages, including non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma.

1.1. Definition

NAFLD is identified among individuals with a minimum of five percent hepatocytes filled with fatty deposits, using either a liver

* Corresponding author. Faculty of Pharmacy, Beni-Suef University, Egypt Ground mail: El Shahed Shehata Ahmed Hegazy St., Beni-Suef, Egypt.
E-mail address: mohamed.omar@pharm.bsu.edu.eg (M.O. Mahmoud).

https://doi.org/10.1016/j.heliyon.2024.e30387
Received 23 August 2023; Received in revised form 4 April 2024; Accepted 25 April 2024
Available online 25 April 2024

biopsy or an imaging assessment in people who consume tiny or no alcohol and do not have any underlying factors contributing to fatty liver disease [2]. NAFLD is categorized into two subtypes: non-progressive (NAFL) and progressive (NASH). A histological assessment is necessary to diagnose NASH in patients suspected of having NAFLD. steatosis is a hallmark sign of it, along with bloating and inflammation of the liver lobes (Fig. 1). Patients diagnosed with NASH face a heightened susceptibility for developing cirrhosis and HCC [3].

1.2. NAFLD prevalence

Approximately one billion people, which is almost a quarter of the world's population, are believed to be affected by NAFLD [4]. NAFLD prevalence shows greater variability across geographies; Middle Eastern and South American countries have the highest rates, while those in Africa have the lowest rates of incidence on the planet [5].

In recent years, NAFLD's prevalence in the U.S. has leaped from 20 % between 1988 and 1994 to 32 % between 2012 and 2016. Even though viral hepatitis incidence has decreased across the United States, there has been a striking rise in the incidence and prevalence of NAFLD. This increase has also been observed in children and youth, making NAFLD a major socio-economic burden worldwide [6].

1.2.1. Prevalence according to age, sex, and ethnic differences

NAFLD exhibits a higher incidence rate among certain age groups and genders. A study found that the yearly incidence rate of NAFLD was 3.5 % for individuals aged 50 years or younger and 5 % among those aged 50 years or older. Among individuals younger than 45–49 years, the age-standardized incidence rate (ASIR) grew dramatically with age, decreased from age 45–49 to 65–69 years, and then increase again for those aged 65–69 years and older [7].

NAFLD has the highest prevalence among males aged 40–49 and among females aged between 60 and 69 [8]. Studies show that, men are more likely to develop NAFLD compared to women of reproductive age, and the difference in prevalence tends to equalize after menopause [9]. To understand the disease's burden, it is important to consider age and gender inequalities in NAFLD prevalence [10].

Numerous studies have highlighted the varying prevalence of NAFLD among various ethnicities. For instance, in the United States, a study revealed that the highest burden of NAFLD was among Hispanic individuals, with a prevalence of 22.9 %, followed by Whites at 14.4 % and Blacks at 13.0 % [11]. Similarly, a study conducted in China revealed that Uyghur ethnics had a considerably higher frequency of NAFLD than Han, Kazakh, or Mongolian ethnic groups [12]. Studies have shown that there is racial and ethnic diversity in the severity of NAFLD among diabetic or prediabetic individuals. Mexican Americans and non-Hispanic Whites have been found to have higher rates of severe NAFLD than other racial/ethnic groups. These findings emphasized the importance of considering race and ethnicity when identifying populations that are more susceptible to acquire NAFLD and when creating focused interventions and health policies [13].

1.2.2. The anticipated future prevalence of NAFLD

Over the next decade, the worldwide incidence of NAFLD is anticipated to rise. A systematic review predicts that by 2040, the worldwide incidence of NAFLD will reach 55.4 %. Significant increases in prevalence are expected in various regions, including Europe, China, and the Middle East [14]. Meta-analysis studies between 1990 and 2019 estimated the global prevalence of NAFLD as 30.1 %. The Middle East, and North Africa (MENA), Latin America exhibit the most elevated prevalence rates of NAFLD. Over the past thirty years, NAFLD prevalence has risen from 25 % to 38 %, indicating a substantial upward trend [15]. These findings highlight the growing global impact of NAFLD and the need for comprehensive approaches to address this public health issue [16].

While NAFLD has become more common globally, it is crucial to remember that the reported prevalence numbers may not be entirely accurate due to the variations in the methodologies used in the studies. These variations include differences in sample size, diagnostic techniques, and dietary and lifestyle practices [17,18]. For instance, while radiologic examination can detect NAFLD, a liver biopsy is necessary to diagnose NASH and determine the extent of fibrosis.

Estimating the real incidence of NAFLD is challenging due to lack of agreed-upon diagnostic standards [17,19]. These discrepancies

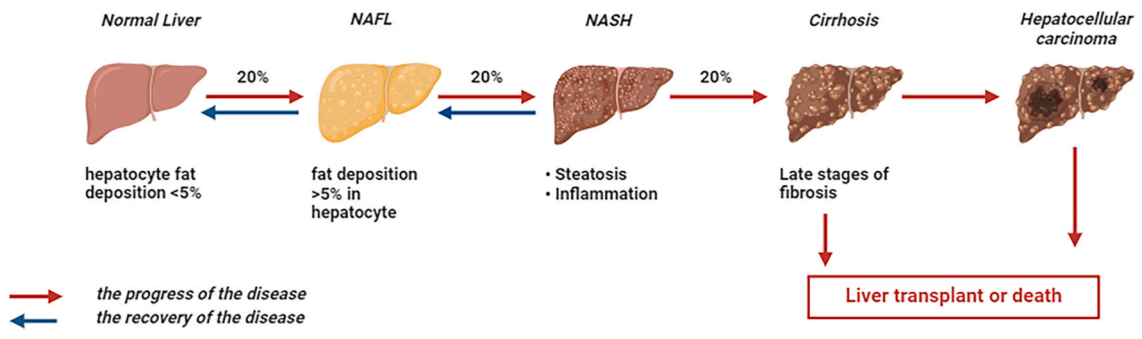


Fig. 1. NAFLD progression from NAFL to liver cirrhosis and HCC.

may contribute to the disparities in NAFLD prevalence estimates among researchers. Therefore, caution should be exercised when comparing prevalence data from various studies, and further research is needed to establish more consistent diagnostic standards and procedures for determining NAFLD prevalence.

1.3. Clinical signs

Most patients with NAFLD have no symptoms, and the condition can remain dormant until cirrhosis develops. The most common symptoms observed among those with NAFLD are fatigue and right upper quadrant discomfort. Echogenic liver on ultrasound may be incidentally observed or as part of right upper quadrant discomfort examination in patients with NAFLD [2]. Liver function tests mirror a hepatocellular model of enzymatic elevation with alanine aminotransferase (ALT) being preferred to serum aspartate aminotransferase (AST). Platelet counts gradually decline with the progression concerning fibrosis and cirrhosis. In individuals afflicted with cirrhosis who exhibit symptoms of synthetic malfunction, serum albumin decreases and prothrombin time increases [20].

1.4. Natural history of NAFLD

Fibrosis progress is significantly faster in NASH than with NAFLD, as it requires only 7 years for each stage of fibrosis to progress, whereas it needs about fourteen years for each stage in NAFLD patients [21].

Only 20 % of individuals with NAFLD are expected to develop NASH, and twenty percent of these cases could build cirrhosis throughout 3 to 4 decades. Individuals with cirrhosis-related NASH are at risk of HCC incidents (Fig. 1). Therefore, regular screening and monitoring of HCC is recommended for those patients, using ultrasound with or without alpha-fetoprotein (AFP) measurements every six months [22].

Among individuals with NAFLD, cardiovascular disorder is the most prevalent cause of mortality, followed by cancer and other liver diseases [23]. A cohort study in Japan found that the mortality rate due to heart disease was ten times elevated in non-obese persons with NAFLD than in individuals who did not have NAFLD [24]. However, as liver cirrhosis progresses, hepatic issues become the primary reason of death. Currently, NASH ranks as the second most prevalent reason for liver transplantation in the United States and may soon become the primary cause. Additionally, NASH-related HCC is rising, and NASH has recently emerged as a primary determinant in the rise of HCC requiring hepatic transplantation [25].

1.5. New nomenclature focused on metabolic factors

The names NAFLD and NASH have been criticized for their stigmatizing language and exclusionary confounding phrases. To address this issue, three international liver groups conducted a modified Delphi process with content experts and patient advocates. The results showed that 74 % of respondents agreed that the current terminology needs improvement while 66 % of respondents found

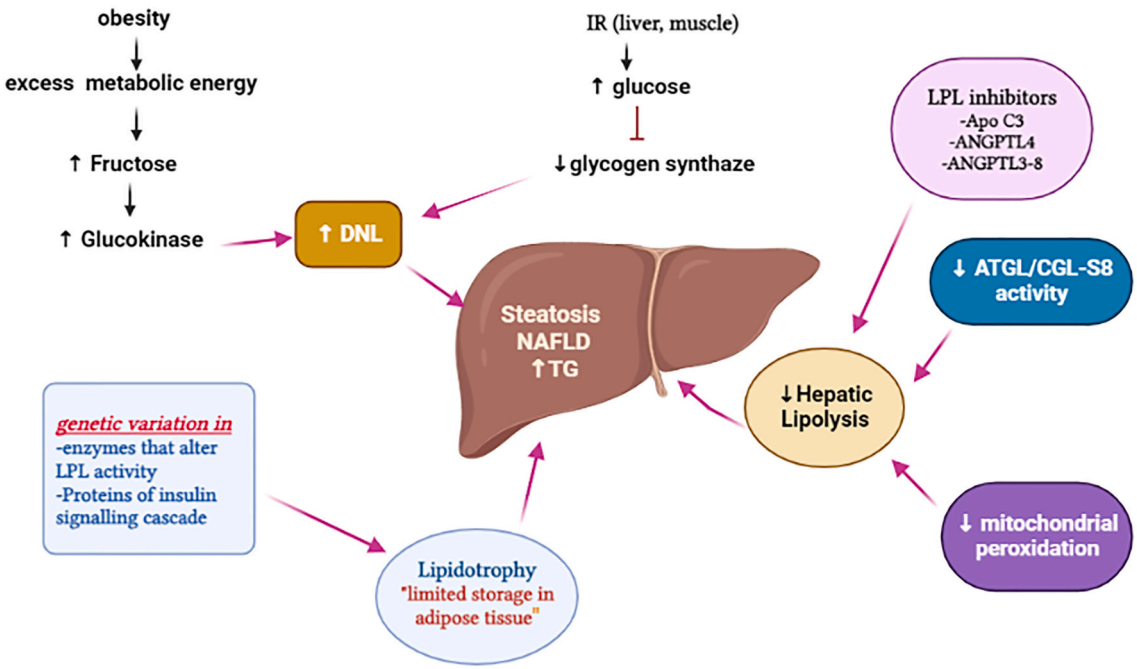


Fig. 2. Metabolic causes of NAFLD. LPL: lipoprotein lipase, IR: insulin resistance, TG: triglycerides, ApoC3: apolipoprotein-C3, ANGPTL4: angiopoietin like 4, ANGPTL 3–8: angiopoietin like 3–8, ATGL: adipose triglyceride lipase, CGL-S8: comparative gene identification-S8.

the labels "fatty" and "nonalcoholic" were stigmatizing. The term "Steatotic Liver Disease" was used to encompass various steatosis etiologies.

NAFLD has been substituted by Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), due to the new definition including a minimum of one of the five cardiometabolic risk variables. In cases where there are no metabolic markers and no recognized cause, the term "Cryptogenic Steatotic Liver Disease" is used.

Metabolic and Alcohol-Related/Associated Liver Disease (MetALD) is used to describe persons who consume more alcohol on a weekly basis and have steatotic liver disease due to metabolic inefficiency. The updated terminology and diagnostic guidelines have gained widespread accepted, non-stigmatizing, and aim to raise consciousness and assist patients [26].

2. Mechanisms of progression of NAFLD and NASH

2.1. Metabolic mechanism's role in the progression of NAFLD

Metabolic mechanisms that lead to NAFLD suggest an imbalance in liver metabolic energy. Energy, such as carbohydrates and lipids, enters the liver based on how efficiently the liver can convert that energy into CO_2 or transport it into very low-density lipoproteins (VLDLs), resulting in the liver storing fuel as triglycerides. This may explain the prevalence of NAFLD in overweight people and people with lipodystrophy, who have inadequate fat storage in their white adipose tissue and store excess energy in their liver [27]. Excessive consumption of any food can provoke NAFLD. Saccharides in the form of mono and disaccharides, particularly fructose and sucrose, can trigger *de novo* liver lipogenesis, which progressively intensifies NAFLD (Fig. 2). Fructose is primarily processed by the liver, so nutritional fructose is directed to the liver and is often converted to triglycerides *via de novo* lipogenesis (DNL) [28]. Individuals who have developed insulin resistance in their livers, inhibiting the activation of glycogen synthase by insulin, may experience a shift of glucose to the lipogenic pathway, promoting the development of NAFLD. The same issue can occur with insulin resistance in skeletal muscle, which can lead to the expansion of NAFLD by increasing liver DNL and triglycerides levels, as ingested glucose is diverted away from glycogen synthesis in skeletal muscle and towards the liver for DNL [29]. In line with this assumption, mice lacking liver glycogen synthase exhibit insulin resistance in the liver, but increase liver lipogenesis and NAFLD [30].

2.2. Adipocyte dysfunction

NAFLD is caused by hereditary lipodystrophies, which result in the liver storing an abnormally high amount of fatty acids due to the lack of adipose tissue. This causes extreme insulin resistance. Numerous genetic researches have shown that adipose dysregulation contributes to the etiology of metabolic disorders. Reduced adipose tissue growth is connected with genetic regions associated with insulin resistance and dyslipidemia [31]. Some of these variants occur in genes that encode enzymes whose products affect the activity of lipoprotein lipase (LPL) (Fig. 2), while others are situated in genes that encode proteins involved in the insulin signaling cascade. Inhibiting factors for LPL activity, such as angiopoietin-like proteins 3/8 (ANGPTL3/8) complex, apolipoprotein-C3 (ApoC3), and angiopoietin-like 4 (ANGPTL4), may encourage the uptake of hepatic triglycerides through chylomicron remnants leading to hepatic steatosis. However, certain factors such as adiponectin and other substances that boost the activity of LPL enzyme in white adipocytes can protect against NAFLD development in animals that are fed a high-fat diet (HFD) [29]. Other metabolic causes of hepatic steatosis include defects in triglyceride export, increased hepatic DNL due to enhanced glucokinase activity, defects in hepatic lipolysis such as reduced ATGL/CGI-58 activity, and decreases in mitochondrial/peroxisomal β-oxidation in the liver [32].

2.3. Visceral adipose tissue (VAT)

Visceral adipose tissue (VAT) has been linked to NAFLD, hyperlipidemia, and insulin resistance. VAT's high lipolytic activity promotes a greater fatty acid shuttle towards the liver, which finally causes insulin resistance, dyslipidemia, and hepatic steatosis. If the capacity of subcutaneous adipose tissue is enhanced, VAT may function as an additional ectopic location for lipids [33]. Therefore, a rise in VAT is not a significant contributor to metabolic imbalance but rather reflects a progression in the biology of subcutaneous white adipose tissue (WAT). As a result, it is not a true distinct hazard element for NAFLD but rather a more noticeable indicator of NAFLD.

2.4. Insulin

Insulin primarily regulates hepatic glucose and fatty acid metabolism in the liver through direct and indirect extrahepatic routes. After a meal, insulin levels increase in the portal vein, which stimulates the activity of hepatic insulin receptor tyrosine kinase (IRTK). This triggers a cascade of intracellular signaling that facilitate glucose disposal [29]. The intracellular signaling pathways consist of mammalian targets of rapamycin complex 2 (mTORC2) and 3-phosphoinositide-dependent kinase-1 (PDK1), which converge on Akt phosphorylation [34]. Insulin blocks hepatic glucose synthesis by activating hepatic insulin receptor kinase, which in turn activates hepatic glycogen synthase and glucokinase and reduces gluconeogenic enzyme activity [35].

Studies have hypothesized that peripheral insulin activity may impede hepatic glucose production by inhibiting WAT lipolysis. This, in turn, decreases hepatic acetyl-coenzyme A (CoA) levels and lowers pyruvate carboxylase (PC) activity and flux (Fig. 3). Additionally, it reduces the transfer of glycerol to the liver, thereby lowering the transformation of glycerol into glucose *via* gluconeogenesis [36].

Insulin affects hepatic glucose metabolism directly *via* the hepatic IRTK/AKT2 pathway, which primarily impacts hepatic glycogen. When hepatic glycogen levels are sufficient, the insulin's direct effects on liver glycogen and glucose metabolism are typically observed after a short period of fasting. However, in states of glycogen insufficiency in the liver, hepatic glucose synthesis is primarily regulated by insulin's indirect effects on glucose metabolism in the liver. This is achieved through the reduction of WAT lipolysis and the suppression of hepatic gluconeogenesis, which becomes the liver's principal source of glucose production [37].

Insulin plays an essential role in controlling the metabolic rates of lipid within the liver through both direct and indirect extra-hepatic pathways. It enhances the activity of mTORC1, which controls the translation of SREBP1c mRNA. Additionally, it triggers SREBP1c activity by increasing mRNA expression and breaking down its precursor protein to produce a full nuclear transcription factor [38,39].

Peripheral insulin activity indirectly controls hepatic lipid production. Hepatic triglycerides synthesis can occur from triglycerides inside lipid molecules (e.g., LDL, CM remnants) and circulating fatty acids when plasma concentrations of ApoC3 rise (Fig. 3). Insulin's impact on all of these inputs is influenced by its effects outside the liver. Insulin promotes LPL activity at peripheral sites such as WAT, resulting in the breakdown of triglycerides through hydrolysis. This process predominantly occurs as VLDL particles, CM, and enhances fatty acid uptake by WAT. Furthermore, Insulin suppresses the lipolysis of WAT and increases the availability of fatty acyl-CoA, which can be converted into triglycerides in the liver *via* esterification. Hepatic triglyceride production *via* esterification is primarily driven by the availability of fatty acids, regardless of alteration in the signaling of hepatic insulin. On the contrary, *de novo* hepatic lipogenesis is insulin-dependent and is impaired in rodents where hepatic insulin signaling is impaired [40,41].

2.4.1. Insulin's role in the regulation of lipogenesis

In response to nutrition and metabolic hormones, as insulin, glucagon, and glucocorticoids, lipogenesis is tightly regulated. Lipogenesis is enhanced during the postprandial stage due to an increase in insulin and serum glucose. This is achieved through signaling cascades that activate lipogenic transcription factors, including liver X receptor (LXR)-α, carbohydrate-response element-binding protein (ChREBP), SREBP1c, and upstream transcription factor. In addition to transcription factors, AKT-mTOR complex controls the insulin signaling cascade, which phosphorylates Ser455 on ACLY to boost its activity [42]. Glucocorticoids inhibit glucose uptake and lipogenesis under conditions devoid of insulin or fasting. On the contrary, in the presence of insulin, glucocorticoids promote lipogenesis by evoking AMPK activity, which stimulates the expression of genes associated with lipogenesis.

2.4.2. Selective insulin resistance

In an obese liver with hyperinsulinemia, high insulin levels promote lipogenesis but do not inhibit gluconeogenesis. This phenomenon is known as "selective insulin resistance." There is speculation that ER stress may stimulate SREBP1c cleavage in the livers of insulin-resistant obese animals, independent of insulin signalling, thus enhancing lipogenesis. Moreover, it seems that signalling pathways operating after the phosphoinositide 3-kinase (PI3K)–AKT axis are responsible for this phenomenon [43,44].

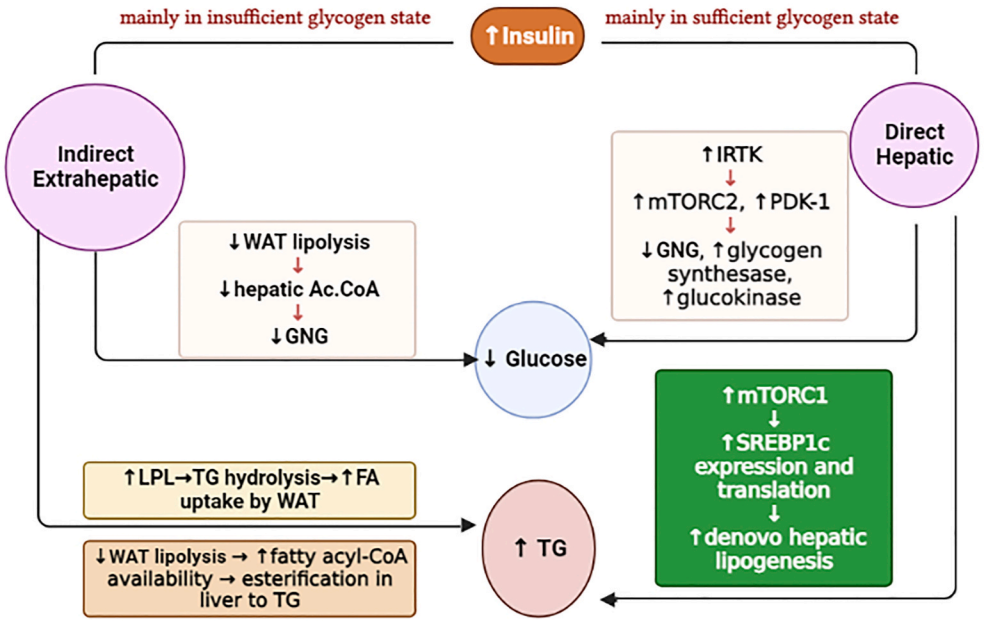


Fig. 3. Role of insulin in the pathogenesis of NAFLD. WAT: white adipose tissue, IRTK: insulin receptor tyrosine kinase, mTORC2: mammalian target of rapamycin complex 2, PDK-1: 3-phosphoinositide-dependent kinase-1, SREBP1c: sterol regulatory element-binding transcription factor 1, GNG: gluconeogenesis.

2.4.3. Insulin gas pedal

According to the "gas pedal" model, insulin regulates hepatic DNL and can accelerate hepatic TG biosynthesis through insulinization or the amount of insulin signal transduction upstream necessary for controlling downstream pathways. This model suggests that insulin's influence on TG biosynthesis remains largely unaltered, regardless of its diminishing capacity to inhibit hepatic glucose production (HGP). According to the selective insulin resistance model, insulinization is required for fewer processes that result in TG accumulation compared to those result in excessive HGP. Promoting TG biosynthesis requires a lower insulinization threshold than maintaining euglycemic HGP. Therefore, in a state of insulin resistance, even moderately elevated or contextually inappropriate insulin levels that persist throughout the day and night in a highly pro-lipogenic metabolic environment can result in significant cumulative biosynthesis of TG. The development of MAFLD is determined by hepatic insulinization, the environment, and timing [45].

3. The inherited nature of NAFLD

The hereditary component of NAFLD and NASH is estimated to be 35–61 % [46]. Constant and powerful familial interaction with a greater risk of progressive disease amongst the closest blood relatives of persons with NASH and the risk rises through monozygotic twins highlight this inherited risk but also suggests a shared gut-microbiome across household persons [47].

Epigenetic alterations have been linked to the inheritance of NAFLD. Differences in their expression account for the higher risk concordance observed in monozygotic compared to dizygotic twins. MiR-30c and miR-331-3p are heritable microRNAs (miRNAs) with comparable predicted target genes. miR-12, miR-21, miR-124a, miR-23b, miR-451a, and miR-34a are other miRNAs associated with NAFLD [48–50].

Early life exposure affects the risk of NAFLD [51]. Research in both animals and human shows a correlation between maternal BMI and hepatic fat levels and lipotoxicity in newborns. Furthermore, markers of developing obesity such as insulin resistance, hyperglycemia, and hyperlipidemia are present in mothers [52]. Placental variables, including oxidative stress, inflammation, and increased lipid and glucose transport, may also contribute to NAFLD's development. These alterations can cause infants to be born with fatty liver disorder that is characterized by lipid buildup, oxidative damage, and immunity problems. The latter are largely due to maternal and infant dietary influences on macrophage phenotype and microbiota [51].

4. Gut microbiota's role in the onset of NAFL disease

Numerous studies support the idea that gut microbiome plays a significant function in NAFLD development [47,53]. As NAFLD and fibrosis progress, the gut microbial populations of afflicted patients become less distinct and have a greater quantity of Gram-negative bacteria and Streptococcus [54]. Certain species such as Bacteroides and Ruminococcus, have been associated with liver diseases such as fibrosis and steatohepatitis [55]. A metagenomic investigation of fecal samples obtained from 56 obese patients diagnosed with steatosis revealed metabolic inconsistencies pertaining to aromatic and branched-chain amino acids. Additionally, there was an increased in number of genes involved in lipid metabolism, hepatic inflammation, and endotoxin production [56]. Studies hypothesize that the gut microbiota contributes to NAFLD through various mechanisms. This include altered energy metabolism, boosted concentrations of aromatic and branched-chain amino acids, reduced microbial diversity, increased production of endotoxins by bacteria, and increased hepatic lipid buildup *in vitro* and *in vivo* [54].

5. Inflammation, injury, and cell signaling in NAFLD

5.1. Nuclear receptors in NAFLD/NASH

Recent research has highlighted the importance of nuclear receptors (NRs) in the development and management of NAFLD and NASH. NRs, including REV-ERBs, ERRs, PXR, PPARs, CAR, HNF4α, and FXR, are crucial regulators of lipid metabolism and inflammation in NAFLD patients. These receptors are considered as master regulators and have resulted in the creation of prospective therapeutic approaches for NASH and NAFLD. Considerable research has been undertaken to elucidate the interrelatedness of liver physiology and metabolism with the receptors that regulate them. Targeting these receptors has showed promising outcomes for treating these conditions. Recent studies have yielded evidence that emphasizes the significance of NRs in the progression and potential therapeutic targeting of NAFLD and NASH [57].

5.1.1. Bile acid signaling and nuclear receptor in NAFLD

Bile acid have been claimed to be of biological and therapeutic interest for NAFLD, as they may cause liver parenchymal diseases and play a regulatory role in hepatic homeostasis [58]. Recent discoveries has solidified bile acids status as multipurpose signaling molecules due to the discovery of receptors such as Takeda G coupled protein receptor-5 (TGR5; GPBAR1, M-BAR), farnesoid X-receptor (FXR), sphingosine-1-phosphate receptor 2 (S1PR2), pregnane X receptor (PXR), vitamin D receptor (VDR), and muscarinic receptors M2/3 [59–61]. TGR5 is a G-protein coupled receptor, while VDR, PXR, and FXR are nuclear receptors triggered by ligands. Numerous investigations have been conducted on FXR due to the ongoing clinical progress of small-molecule FXR ligands. Specific TGR5 agonists such as (obeticholic acid and baricitinib) and dual TGR5- FXR agonists such as (INT-777) have been shown to diminish hepatic steatosis. The therapeutic value of FXR agonists has been linked to the initiation of its subsequent effector, FGF15/19. Activation of FXR affects energy metabolism, lipid and glucose homeostasis, hepatic inflammation, and cellular stress. Additionally, it promotes the uptake of intestinal bile acids [62]. Even though some effects of FXR agonists are opposite in rodent models and humans,

their overall therapeutic efficacy may be attributed to their effect in the intestine. This may account for the species-specific variances. Furthermore, TGR5 agonists induce the secretion of glucagon-like peptide-1 (GLP-1) from the intestine. TGR5 agonists also manifest pleiotropic effects *via* signaling in the liver stellate cells, macrophages, and sinusoidal endothelium [63]. Additionally, the liver x receptor (LXR) has been associated with NAFLD due to its significant impact on lipid and glucose homeostasis. In mice, the loss of LXR improves insulin sensitivity but increases glucose intolerance. However, LXR agonists (GW3965 and LXR-623) have been found to increase hepatic fat making them unsuitable treatments for metabolic syndrome due to this side effect [6].

5.1.2. Estrogen-related receptors (ERRs) in NAFLD

ERRs are primary regulators of liver physiology and metabolism. The biology and characterization of these nuclear receptors have facilitated the synthetic ligands creation. The potential therapeutic utilization of these receptors to address NAFLD appears promising, given the ongoing clinical trials of various compounds such as cilofexor, thiazolidinediones, and saroglitazar [57]. Estrogens are particularly necessary for the growth and operation of the reproductive system, especially in females. ER mediates the actions of estradiol (E2), a traditional estrogenic hormone. One of the two ER isoforms capable of binding to estrogen and altering gene expression is ERα. ERα exhibits a tissue-specific binding pattern and can bind to DNA either directly or indirectly through other transcription factors. In the liver, ERα primarily collaborates with AP-1 to regulate genes involved in the metabolism of fats and carbohydrates, such as SHP and STAT3. STAT3, which is a direct target of ERα, mediates the hepatoprotective effects of estrogen. In mice, the loss of ERα cause fatty liver disease, and estrogen influences liver metabolism through extrahepatic tissues [64].

5.2. NAFLD-related inflammation and immune system signaling

The pathogenesis of NAFLD and NASH has been linked to an interconnected network of inflammatory and immunological signaling pathways. These pathways include the induction of inflammasomes, hepatocyte-derived signaling, changes in signals of the innate immune system, altered macrophages and platelets, the activation of T cells and the production of neutrophils, and changes in adipokines, cytokines, and chemokines. The significance of these factors and their participation in the progression of NASH is unclear, as is the case with other factors involved in NASH etiology [65].

Many different cell types, both resident and invading, are involved in inflammasome stimulation in NASH. Caspase-1 is activated by the multiprotein complex in response to damaging signaling, such as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), which then splits and triggers interleukin-1β and its subfamily members IL (a, 18, and 33) all of which augment NASH characteristics such as intercellular adhesion molecule 1 (ICAM-1) stimulation on sinusoidal endothelium, metabolic disorder, and activation of stellate cells and subsequent fibrosis [66]. The nucleotide-binding domain and leucine-rich repeat pyrin domain containing 3 complexes (NLRP3) is the most well-documented inflammasome in NASH cases where blocking it in myeloid cells reduces the inflammatory state and fibrosis in an experimental model using mice [67]. In both human and animal studies of steatohepatitis, a subversive caspase substrate known as gastrin-D leads to hepatocyte death *via* inflammation [68].

5.3. Liver macrophages in NAFLD/NASH

Liver macrophages (Kupffer cells) have been extensively studied due to their phenotypic diversity and relevance in the histopathology of NASH. They secrete IL-1β, monocyte chemoattractant protein-1 (MCP-1), and tumour necrosis factor (TNF-α), which contribute to the progression of fibrosis and inflammation [69]. However, the polarization of macrophages in the liver is not well understood. However, it is understood that these cells undergo metabolic reprogramming upon activation after damage. This process is marked by the activation of the inflammasome, which boosts the synthesis of reactive oxygen species and lactate [70].

5.4. T-cell and NASH

There is a correlation between the advancement of NASH and abnormal T-cell responses. Inflammatory and metabolic features of NASH are contributed by IL-17 secreting TH17 cells. Those with NASH have a higher quantity of liver Th17 cells and a decrease in dormant Tregs (Tregs; $CD4^{+}$, $CD45RA^{+}$, $CD25^{++}$) as well as elevated concentrations of $IL\text{-}4^{+}$ and $IFN\text{-}\gamma^{+}$ cells in the bloodstream. Individuals with NASH have elevated levels of natural killer (NK)-T cells in their livers. Experimental studies have shown that reducing these cells can alleviate symptoms of NASH [71].

5.5. Neutrophils and their role in the pathogenesis of NASH

Until recently, neutrophils received little attention. However, their emerging role as mediators of fibrosis resolution and tissue inflammation highlights their recent significance in the pathogenesis of NASH [65]. Neutrophils are part of the specific pro-resolving mediator system (SPM), which consists of four key stages: resolution of prolonged injury, transition from an inflammatory state to resolution, inactivation of myofibroblasts, and degradation of extracellular matrix [72]. A cascade of lipid mediators, including protectins, maresins, and resolvins, is set in motion by several enzymatic processes. The processes involved the metabolism of docosapentaenoic acid, arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid. Interestingly, the same lipid mediators that are linked to SPM have been found to be possible indicators that can distinguish between NAFLD and NASH and determine the fibrosis stage. This offers more evidence to support the idea that NASH is a condition of impaired pro-resolution [73]. Recent studies have highlighted the involvement of neutrophils in the pathogenesis of NASH [74]. In a model of NASH induced by diet, mice deficient

in mitogen-activated kinases, p38g, and p38d, in myeloid cells exhibited reduced disease characteristics. This was due to the decreased migration of neutrophils lacking p38 g/d to the injured liver. Previous studies have shown that p38g and p38d exhibit increased levels in the livers of patients with NASH, indicating a potential role in the disease [75]. The resolution of fibrosis may be facilitated by neutrophils because of their potential to induce macrophage polarization towards a restorative, matrix-degrading phenotype. This impact is aided by neutrophils delivering miR-223 to macrophages *via* extracellular vesicles, which reduces the production of mRNA that encodes the scaffold protein for the Nlrp3 inflammasome [76].

5.6. Platelets in NAFLD/NASH pathogenesis

Platelets have the potential to induce inflammation and damage to hepatocyte through the action of GPIba, a von Willebrand factor membrane receptor. In NASH pathogenesis, platelets require Kupffer cells to play their role, and thrombin or fibrinogen to promote NASH in the first place. In animal models of NASH, platelets activation and adherence, but not aggregation, are involved in the pathogenesis. These findings support the use of currently available platelet antagonists in preventing and treating NASH and NASH-associated HCC [6].

5.7. The role of TREM2+ macrophages in the progression of NASH

Macrophages, a specific subset of immune cells, express triggering receptors expressed on myeloid cell 2 (TREM2) protein. TREM2+ macrophages have been linked to a vital function in the pathophysiology of NASH. Studies have indicated that TREM2+ macrophages are attracted to fibrotic regions of the liver and contribute to slowing down of NASH progression [77]. It has been proposed that TREM2+ macrophages inhibit the production of lipopolysaccharide (LPS)-loaded lipoproteins, which in turn induce a pro-inflammatory response. This indicates that TREM2+ macrophages have a regulatory role in controlling inflammation during the progression of NASH [78].

Macrophages that express TREM2+ slow the progression of NASH by suppressing the secretion of pro-inflammatory cytokines like IL-6. They also reduce hepatic inflammatory responses through several different pathways, which is critical for limiting the progression of NASH. When Trem2 is deficient in hematopoietic cells, it results in impaired lipid processing and extracellular matrix modification, leading to intensified steatohepatitis, fibrosis, and cell death. TREM2+ macrophages are crucial in phagocytosing dying hepatocytes, which is essential for resolving inflammation and preventing NASH progression. This highlights the significance of TREM2+ macrophages in halting the advancement of NASH [77–79].

5.8. B cells in NASH pathogenesis

Recent studies have emphasized the growing importance of B cells in the pathophysiology of NAFLD and its advanced form, NASH. B cells have been associated with numerous autoimmune and inflammatory illnesses. Research indicates that patients diagnosed with NAFL or NASH exhibit increased levels of intrahepatic B lymphocytes, particularly in the portal tract, indicating their role in the inflammatory process of the portal wall. In rodent models of NASH, the livers of mice with NASH have been shown to accumulate more B lymphocytes. These cells express proinflammatory gene expression profiles and release higher amounts of IgG, which stimulates inflammation and fibrogenesis. The role of B cells in NAFLD and NASH is harmful, as evidenced by the enhanced glucose tolerance and decreased liver fibrosis displayed by B-cell-deficient mice [80].

B cells stimulation in NAFLD is caused by elevated oxidative stress and hepatic production of B-cell-activating factor. Studies have shown that B cells play a significant role in the pathophysiology of NAFLD and NASH by directly promoting inflammation, localized fibrogenesis, and insulin resistance in the liver [81].

There is a growing interest in investigating B cell-specific therapy methods due to the increasing data on the role of B cells in the NAFLD progression, including their activation mechanisms and possible contribution to NASH-associated HCC. Several strategies such as B cell exhaustion, inhibition of B cell survival and impairment of B cell proliferation, manipulation of B cell receptor signaling, vaccination, and techniques that target B-cell mediators of inflammation like TNF-α have been proposed [82].

In brief, ongoing research is investigating the function of B cells in the occurrence of NAFLD and NASH. Further research is necessary to develop targeted treatment strategies and clarify the precise processes by which B cells are involved in the pathophysiology of NAFLD and NASH.

5.9. IgA^+ plasma cells in NAFLD/NASH

Research has shown that IgA^+ plasma cells may have an impact on NAFLD and its progressive form, NASH. Individuals with NASH have been found to have a higher concentration of IgA-producing plasma cells in their liver. These cells express proinflammatory gene expression profiles and emit higher amounts of IgG, which in turn promote inflammation and fibrogenesis [81,82].

Furthermore, it has been demonstrated that IgA^+ cells residing in the liver hinder the protective function of $CD8^+$ cytotoxic T lymphocytes, leading to HCC in NASH patients.

The precise contribution of IgA^+ plasma cells to the pathophysiology of NAFLD and NASH remains unclear. However, these cells may promote fibrogenesis and liver inflammation by suppressing $CD8^+$ cytotoxic T cells and secreting proinflammatory cytokines [83].

Further inquiry is required to fully clarify the precise mechanisms and ramifications of IgA^+ plasma cells in relation to NASH and

NAFLD.

5.10. CXCR6+ T cells in the progression of NASH

CXCR6+ T cells, an emerging subset of immune cells, have garnered considerable interest in recent times due to their possible involvement in the advancement of NASH. They are a subclass of $CD8^+$ T cells that express the surface protein CXCR6, which facilitates their interaction with CXCL16. Research indicates that CXCR6+ T cells aggregate in the NASH patients's liver [84].

These cells exhibit an activated and fatigued phenotype, indicating that despite constant stimulation, they ultimately fail to eliminate the damage. They appear to specifically target and harm healthy liver cells, thereby exacerbating inflammation and fibrosis [85].

Additionally, CXCR6+ T cells may lack antigen specificity, suggesting that they selectively target liver cells rather than specific foreign invaders, as conventional T cells do [86].

5.11. Hepatocyte oxidative stress in nonalcoholic fatty liver disease

In NASH, the oxidative condition of hepatocytes can affect intracellular signaling. Mitochondrial injury produces DAMPs that have the ability to stimulate stellate cells [87]. Obesity is associated with the deactivation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) protein tyrosine phosphatase in hepatocytes, resulting in activated STAT-1 and STAT-3 signaling in animal and human NASH models [88]. In this context, the study shows distinct effects for STAT-1 and -3. STAT-1 is involved in the infiltration and fibrosis of T cells, while STAT-3 promotes cancer progression. Additionally, signaling by the nuclear factor of activated T cells (NFATc4) declines PPAR-α [89], which reduces fatty acid oxidation, increases the release of osteopontin 1, and stimulates stellate cells [90,91].

Hepatocytes may play a role in the systemic consequences of obesity, specifically inflammation and insulin resistance. Their production of dipeptidyl peptidase 4 (DPP4) induces insulin resistance and inflammation in visceral adipose tissue by triggering inflammatory signaling in macrophages. This underscores the significance of the interaction between adipose tissue and hepatocytes [92]. Similarly, b-Klotho, which acts as a co-receptor for FGF15/19 and FGF21, regulates the transformation of brown fat by altering the production of bile acids from the liver to the intestine. This lead to changes in the gut microbiota that affect signaling *via* the intestinal bile acid receptor TGR-5 and ultimately modifying thermogenesis [93]. Obesity-induced inflammation can cause hepatic injury due to systemic aspects of the metabolic syndrome. This is because inflammation is linked to hepatocyte expression of protein tyrosine phosphatase receptor gamma (PTPR-γ) which antagonizes insulin action [94]. Additionally, signals from adipose tissue can affect liver function and even cause diseases. Neuregulin-4, a member of the epidermal growth factor (EGF) family released by adipose tissue prevents hepatocytes from undergoing programmed cell death [95].

5.12. Epigenetic modulators of NAFLD and NASH

Hepatocyte epigenetic regulators may aid in the development of NASH. In addition to miRNAs, the genetic deletion of G-protein suppressor 2 (GPS-2), a co-factor in the HDAC3 repressor complex, increases experimental NASH. Its removal reduces illness *via* derepression of PPAR-α [96]. These variants may represent possible therapeutic targets [97].

5.12.1. DNA methylation

DNA methylation is a significant epigenetic alteration that has a vital function in the regulation of genes. It regulates gene expression by binding proteins that inhibit genes or by inhibiting the binding of transcription factors. The interaction of environmental and genetic factors in the development of complex diseases, including the role of DNA methylation, is an area of active research with significant implications for the prevention and treatment of complex diseases [98].

DNA methylation, particularly at CpG dinucleotides, is a common epigenetic modification that can significantly affect gene transcription. *De novo* DNA methylation is primarily carried out by DNMT3A and 3B, while DNMT1 is responsible for maintaining methylation patterns. TET dioxygenases are responsible for DNA demethylation. Gene silencing is often associated with DNA hypermethylation on gene promoters, while DNA hypomethylation usually indicates a transcriptionally active state [99].

DNA methylation is associated with metabolic, neurological, immunological, and cancerous illnesses. High levels of LDL and triglycerides, which are risk factors for metabolic diseases, have been found to be linked to DNA methylation. Obesity and NAFLD are complex metabolic illnesses caused by both environmental factors and genes, which are linked through epigenetic mechanisms [98].

The field of study regarding DNA methylation is still in development, and there is much to learn about its effects on lipid metabolism and the onset of fatty liver disorders. The increase in DNMT1 expression in NAFLD may explain the alteration in DNA methylation. NAFLD may be develop and worsen due to increased CpG methylation of genes responsible for fatty acid oxidation and decreased methylation of genes responsible for fibrosis compared to healthy individuals. A study of liver biopsy samples from individuals with NAFLD revealed a negative association between methylation levels at the promoter region of the PGC1$\boldsymbol{\alpha}$ gene, which controls the process of fatty acid oxidation, and its expression. Furthermore, a significant direct correlation was found between methylation levels and insulin resistance [100].

5.12.2. MicroRNAs (miRNAs)

MiRNAs are short RNA molecules, ranging from 21 to 25 bases in length that are endogenous and have been present in evolutionary

history since the development of sponges. The quantity of miRNAs has grown in correlation with the level of complexity, with an estimated 2500 miRNAs present in the human genome. MiRNAs are important in the epigenetic control as they can control multiple genes or influence a single gene. They attach to and break down the 3-UTR region of discrete mRNAs, thereby controlling the expression of specific genes.

According to recent studies, miRNAs play a significant role in the pathogenesis and progression of NAFLD. The mechanisms behind this have gained much interest. The potential contribution of dysregulated miRNA targets implicated in fibrotic oxidative stress, inflammation, and lipid metabolism to the development and progression of NAFLD cannot be ruled out. MiRNAs can regulate both intracellular and intercellular gene expression as they are exocytosed into extracellular vesicles (EVs) and are capable of influencing other cells [101].

5.13. Injury and death of hepatocytes

Hepatocyte inflating is a key histologic feature of severe hepatic injury and is closely correlated with the severity of NASH. Understanding the pathways of hepatocyte damage and death is critical for comprehending the etiology of the illness. The cellular degeneration in these swollen hepatocytes, also called "undead cells," may augment inflammatory and fibrotic signals in the pericellular space [102]. The progression of NASH is accompanied by a spectrum of reactions and mediators triggered by a gradient of cell death pathways [73]. There are three distinct types of cell death involved in these processes: apoptosis, necroptosis, and pyroptosis. Additionally, it has been proposed that sublethal lipotoxic hepatocyte injury affects hepatocyte function and increases illness without leading to full cell death [102]. Autophagy is a homeostatic response that maintains cellular energy levels. However, it may contribute to cell death in case of failure in its prevention. Several apoptosis mediators have been identified in human and animal studies of NASH, including caspase-2, caspase-8, apoptosis signaling regulated kinase-1 (ASK-1), CASP8 and FADD-like apoptosis regulator (CFLAR), and TNF-related apoptosis-inducing ligand (TRAIL) [103]. ASK-1 is highly expressed in the livers of individuals with NASH. This leads to increased downstream effectors of mitogen-activated protein kinase (MAPK) and p38 activities, which is promoted by TNFAIP3, a deubiquitinating enzyme. NASH can be mitigated in rats and apes by a peptide resembling CFLAR that blocks ASK-1 dimerization [104, 105]. Removing the death receptor TRAIL, which plays a role in cell injury, reduces inflammation within a liver disease model in animals but does not affect fat tissue [105]. TRAIL receptor 2 enhances the pro-inflammatory phenotype of macrophages by promoting extracellular vesicle production from hepatocytes due to harmful lipids and proteins [106]. The contents of these vesicles may also worsen liver damage in NASH [102]. Caspase-2 is activated in experimental NASH by endoplasmic reticulum (ER) stress and TNF-α. This leads to the proteolytic stimulation of site 1 protease (S1P). Deactivating caspase-2, either genetically or pharmacologically, reduces NASH by preventing the activation of SREBP that boosts triglycerides production, raises energy spending, and enables AMPK action [107].

6. The association between nonalcoholic steatohepatitis and hepatocellular carcinoma

The high prevalence of NASH leads to higher rates of HCC [108]. Delayed diagnosis of HCC in NASH can be attributed to two main causes. Firstly, NASH is often undiagnosed or misdiagnosed as "cryptogenic cirrhosis" where HCC is the initial symptom. Secondly, unlike other liver diseases like HBV and/or HCV, where cirrhosis affects 90 % of patients, a larger proportion (35–50 %) of tumors arise before cirrhosis in people with NASH [109]. Due to the higher tendency for tumors to occur before cirrhosis, many individuals may not be aware that they have liver disease. Even those who have been diagnosed with NASH are not regularly examined for HCC until cirrhosis has developed, which potentially too late. Therefore, the inferior results for HCC in the NASH group may be due to delayed diagnosis rather than underlying changes in tumor biology [110].

Due to the distinctive clinical characteristics of HCC in NASH, there is an urgent need for improved investigation procedures to distinguish underlying NASH and enhance screening techniques for HCC. These techniques should not depend on the recognizing signs of cirrhosis to identify high-risk individuals and reduce the ongoing loss of early HCC [111]. Several attempts have been made in recent years to develop more accurate screening methods for detecting circulating tumor cells, DNA, or specific epigenetic modifications using non-invasive technologies. However, none of these methods have been sufficiently validated for clinical use [112].

The higher rates of HCC development before cirrhosis in people with NASH compared to those with other causes suggest the presence of systemic or metabolic risk factors that are specific to NASH. Additionally, NASH not only poses a risk of HCC as the primary cause but is also often overlooked as a contributing factor to HCC in individuals with other causes, as hepatitis C, hepatitis B, and alcohol-related liver disease [63].

Additional research is necessary to examine potential hereditary factors that elevate the risk of HCC in individuals with NAFLD [113]. Currently, the PNPLA3 mutation is the most well established genetic risk factor for NASH, especially in obese individuals [114].

Although the exact causes of NASH remain a mystery, it is clear that the obesity and metabolic syndrome, which often accompany it, pose independent risks beyond those associated with the degree of liver disorder. Indeed, Obesity increases the probability of developing any tumor and represents the most significant risk for HCC [115]. Furthermore, type II diabetes carries a significant risk of HCC in patients with NASH-associated cirrhosis [116]. Metabolic dysregulation and obesity can lead to DNA damage, oxidative stress, and other harmful conditions [117].

6.1. NAFLD/NASH-associated HCC pathways

Hepatocyte lipid aggregation is a hallmark of both NAFLD and NASH and has been linked to several pathogenic drivers of

carcinogenesis, including oxidative DNA damage [118]. Hepatic steatosis is the foundation for oxidant production, which in turn stimulates NASH, fibrosis, and HCC by oxidizing a particular phosphatase that normally deactivates STAT-1 and STAT-3 [88]. Oxidative stress-induced DNA damage may increase the expression of DNA-dependent protein kinase (DNA-PK), leading to inaccurate DNA repair and potential resistance of cancer cells to standard treatment [119].

In an animal study, it was found that increased dietary cholesterol worsen hepatic steatosis, accelerates the progression of HCC, encourages tumor development, and stimulates oncogenic signaling, analogous to what is seen in human NASH-HCC [120]. Additionally, increased expression of the squalene epoxidase enzyme results in elevated levels of cholesterol ester and $NADP^{+}$ and epigenetically inhibits the PTEN tumor suppressor, allowing oxidative stress and cell growth in individuals with NASH and NASH-related hepatic carcinoma. Terbinafine, an antifungal medication approved for clinical use, has been shown to reduce HCC in animal models by inhibiting squalene epoxidase [121]. The biological clock, which is regulated by the opposing actions of CAR and FXR, may surprisingly influence hepatic carcinogenesis in NASH. Several metabolic processes, such as mitochondrial biogenesis, gluconeogenesis, the antioxidant response, fatty acid oxidation, and DNL, can be altered by PGC-1a dysregulation, which has been linked to NASH-HCC development [122]. Steatosis causes hepatocytes to undergo apoptosis and further promotes carcinogenesis in animal models of NASH. The role of apoptosis in HCC remain uncertain due to the carcinogenic effects of the apoptosis-antagonizing transcription factor (AATF) in animal studies [123,124]. Autophagy plays a crucial role in maintaining cellular health by removing lipids and proteins in both normal and steatotic hepatocytes [125]. However, reduced autophagy caused by lipid buildup can upregulate mTOR, potentially leading to promoted hepatic oxidative stress. The connection between decreased autophagy and fatty aggregates in NAFLD and elevated ER stress may encourage inflammation triggered by TNF-α [126]. Autophagy is stimulated by oxidative stress, which expands the nuclear factor erythroid 2-related factor (NRF2) transcriptional activity to produce an anti-oxidant reaction that promotes the survival of cells, including steatotic hepatocytes and cancerous ones [127].

Recent research has emphasized the significance of gut microbiota in the development of hepatic carcinoma [128,129]. Furthermore, it plays a significant part in NASH pathogenesis [130,131]. A distinct gut microbiota has been linked to NASH-related HCC due to the production of bacterially generated deoxycholate, which stimulates a senescence-related secretome that triggers liver inflammation [132]. The liver's antitumor response controlled by NK-T cells may be regulated by the process of converting primary bile acids into secondary ones in the gut and by boosting the production of FGF19, which promotes hepatocyte division. Additionally, stimulation of liver TLR4 and TLR9 signaling may further amplify these effects [133]. While most research on NASH-related HCC focuses on hepatocytes and their dysregulated signaling, it is important to consider the role of other local and invading cells in the liver that also contributes to carcinogenesis. TLR4 signaling triggers inflammation and fibrogenesis in Kupffer and stellate cells, respectively [130,134].

7. Emerging research areas and potential future interventions for NAFLD and NASH

Although a definitive cure for NAFLD/NASH is still to be developed, ongoing research offers numerous promising avenues for managing and potentially treating the condition in the future. The combination of lifestyle modifications with emerging interventions holds significant potential to improve patient outcomes.

7.1. Novel treatment for NASH

7.1.1. Pegozafermin

The researcher's team at the University of California discovered a substance that reduces liver fibrosis and inflammation in NASH patients. This substance exhibits analogue properties to fibroblast growth factor 21 (FGF21), an endogenously synthesized peptide hormone secreted by the liver. FGF21 regulates hepatic lipid metabolism and energy expenditure. Previous research has also demonstrated its ability to reduce blood glucose levels, insulin, liver obesity, and body weight. In addition to ameliorating inflammation, fibrosis, and liver injury, the study also indicated that the novel potential treatment significantly enhances many non-invasive biomarkers of NASH activity and scarring. The randomized 24-week clinical trial assigned 222 NASH patients to take either the drug or a placebo. Approximately 27 % of patients who took higher doses of the drug showed improvement in liver fibrosis, compared to 7 % of patients who took the placebo [135].

7.1.2. Semaglutide (SAMARA study)

Another trial study, known as the SAMARA Study, aims to investigate whether semaglutide, an FDA-approved medication commonly used to treat obesity and type 2 diabetes, is a viable treatment alternative for patients with liver fibrosis resulting from NAFLD.

Semaglutide belongs to the glucagon-like peptide-1 receptor agonist (GLP-1 RA) class of drugs, which mimics the GLP-1 hormone secreted by the gastrointestinal tract after food consumption. In type 2 diabetes, this works by increasing insulin release from the pancreas after meals and decreasing blood sugar levels. Additionally, it slows down stomach emptying leading to a feeling of fullness and lower food intake. Furthermore, delaying glucagon breakdown further aids blood sugar control [136].

7.1.3. Resmetirom

Resmetirom is a selective, orally active, liver-directed thyroid hormone receptor-β agonist currently undergoing phase III clinical studies as a prospective therapeutic intervention for nonalcoholic steatohepatitis. Its effect is exerted by stimulating a particular type of thyroid hormone receptor (β) predominantly located in the liver. Furthermore, it improves hepatic fat metabolism, decreases

lipotoxicity, Reduces the energy production and mitochondrial function in liver cells, and mitigates hepatic inflammation and cellular demise [137].

Till now, the U.S. FDA has not approved any pharmaceuticals for NASH treatment.

7.2. Weight loss and lifestyle modifications

The American Liver Foundation (ALF) recommends weight loss as the initial standard of care for NAFLD and NASH. This can be achieved *via* exercise, calorie restriction, and a balanced diet. Gradual weight loss, typically ranging from 7 to 10 % over a specified period, is advised to reduce hepatic inflammation and fat accumulation. Fasting for rapid weight loss is not recommended as it may worsen NAFLD. Regular physical exercises and a healthy diet are essential for managing NAFLD and NASH [2].

7.3. Supplements and medications

While there is currently no authorized drug for the treatment for NASH or NAFLD, researchers are exploring various drugs that may be effective in resolving these conditions. Vitamin E supplements and specific medications, such as pioglitazone and statins, have shown promise in mitigating liver inflammation and fibrosis in individuals diagnosed with NAFLD/NASH [138].

7.4. Non-invasive diagnostic tests

Ongoing research aims to develop non-invasive diagnostic tests that are both precise and economically viable for identifying NASH in individuals with NAFLD. This is essential for initiating treatment to lower the risk of fibrosis and other NASH-related complications. Currently, NASH can only be identified with certainty *via* an invasive liver biopsy, which presents obstacles to its widespread implementation and subsequent evaluations [139].

8. Novel technologies or methodologies that are shaping research in this field

Recent technological and methodological advancements are impacting research on NAFLD and NASH. These new technologies and methods include.

8.1. Artificial Intelligence (AI)

Artificial Intelligence is being used to create non-invasive NAFLD and NASH diagnosis methods. To diagnose and track NAFLD and NASH, for example, researchers are utilizing machine learning algorithms to examine medical pictures and find trends, analyzing vast datasets to identify disease patterns, predict progression, and optimize treatment strategies [140].

8.2. Omics technologies

Omics technologies, such as metabolomics, proteomics, and genomics, are being utilized to identify biomarkers for NAFLD and NASH. These tools can assist in discovering new targets for pharmaceutical research and enhance patient selection for clinical studies [140].

8.3. Advanced imaging methods

Patients with NAFLD and NASH are being evaluated non-invasively for liver fibrosis and inflammation using advanced imaging methods such as magnetic resonance elastography (MRE) and transient elastography (TE). These methods can assist in identifying people who need to be closely monitored because they are at risk of worsening their liver disease [141].

9. Conclusions

Although there are many obstacles to overcome in the fight against the worldwide epidemic of NAFLD and its associated metabolic and hepatic consequences, there is a solid and systematic foundation upon which to build effective treatments. NAFLD is prevalently present before the definite beginning of NASH, hepatic fibrosis, and hepatic cirrhosis. Improving non-invasive techniques for detecting NAFLD and its stages, including NAFLD/NASH/NASH-related fibrosis, and HCC and developing improved methods to determine who is at risk for NAFLD, particularly among adults who are not fat, remain challenging areas of research. Liver biopsy is currently the gold standard for determining whether or not a potential treatment for nonalcoholic steatohepatitis (NASH) is effective. Preclinical research has shown, however, that other liver issues, including inflammation, fibrosis, and even cirrhosis, can be resolved if metabolic treatments are successful in reversing hepatic steatosis, providing a foundation and road map for formulating novel plans to investigate the disruption of metabolism regarding NAFLD and NASH. Even though these metabolic flaws are not the only deficiencies in this disease, they do contribute to the various stages of illness progression. Therefore, targeting them is likely to be a preliminary part of any effective therapeutic process. Therefore, we hypothesize that combining various medication targeting different metabolic pathways can improve treatment response. This can not only lead to the cure of NASH and a reduction in the progression of fibrosis but also

decrease the risk of other associated diseases and future cancer risk.

Funding sources

This research did not get any dedicated support from public, commercial, or not-for-profit organizations.

CRediT authorship contribution statement

Ahmed M. Samy: Writing – original draft, Resources, Data curation. **Mohamed A. Kandeil:** Supervision, Conceptualization. **Dina Sabry:** Supervision, Conceptualization. **A.A. Abdel-Ghany:** Supervision. **Mohamed O. Mahmoud:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] V.T. Samuel, G.I. Shulman, Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases, Cell Metab. 27 (2018) 22–41.
[2] N. Chalasani, Z. Younossi, J.E. Lavine, M. Charlton, K. Cusi, M. Rinella, S.A. Harrison, E.M. Brunt, A.J. Sanyal, The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases, Hepatology 67 (2018) 328–357.
[3] P.S. Dulai, S. Singh, J. Patel, M. Soni, L.J. Prokop, Z. Younossi, G. Sebastiani, M. Ekstedt, H. Hagstrom, P. Nasr, Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis, Hepatology 65 (2017) 1557–1565.
[4] Z.M. Younossi, Non-alcoholic fatty liver disease–a global public health perspective, J. Hepatol. 70 (2019) 531–544.
[5] C. Estes, H. Razavi, R. Loomba, Z. Younossi, A.J. Sanyal, Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease, Hepatology 67 (2018) 123–133.
[6] R. Loomba, S.L. Friedman, G.I. Shulman, Mechanisms and disease consequences of nonalcoholic fatty liver disease, Cell 184 (2021) 2537–2564.
[7] Y. Lin, X. Feng, X. Cao, R. Miao, Y. Sun, R. Li, J. Ye, B. Zhong, Age patterns of nonalcoholic fatty liver disease incidence: heterogeneous associations with metabolic changes, Diabetol. Metab. Syndr. 14 (2022) 181.
[8] A. Nagral, M. Bangar, S. Menezes, S. Bhatia, N. Butt, J. Ghosh, J.H. Manchanayake, M. Al Mahtab, S.P. Singh, Gender differences in nonalcoholic fatty liver Disease, Euroasian J. Hepato-Gastroenterology 12 (2022) S19.
[9] A. Lonardo, F. Nascimbeni, S. Ballestri, D. Fairweather, S. Win, T.A. Than, M.F. Abdelmalek, A. Suzuki, Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps, Hepatology 70 (2019) 1457–1469.
[10] S. Fresneda, M. Abbate, C. Busquets-Cortés, A. López-González, P. Fuster-Parra, M. Bennasar-Veny, A.M. Yáñez, Sex and age differences in the association of fatty liver index-defined non-alcoholic fatty liver disease with cardiometabolic risk factors: a cross-sectional study, Biol. Sex Differ. 13 (2022) 1–16.
[11] N.E. Rich, S. Oji, A.R. Mufti, J.D. Browning, N.D. Parikh, M. Odewole, H. Mayo, A.G. Singal, Racial and ethnic disparities in nonalcoholic fatty liver disease prevalence, severity, and outcomes in the United States: a systematic review and meta-analysis, Clin. Gastroenterol. Hepatol. 16 (2018) 198–210.
[12] K. Riazi, M.G. Swain, S.E. Congly, G.G. Kaplan, A.-A. Shaheen, Race and ethnicity in non-alcoholic fatty liver disease (nafld): a narrative review, Nutrients 14 (2022) 4556.
[13] M. Shaheen, K.M. Schrode, M. Tedlos, D. Pan, S.M. Najjar, T.C. Friedman, Racial/ethnic and gender disparity in the severity of NAFLD among people with diabetes or prediabetes, Front. Physiol. 14 (2023) 1076730.
[14] M.L.P. Teng, C.H. Ng, D.Q. Huang, K.E. Chan, D.J.H. Tan, W.H. Lim, J.D. Yang, E. Tan, M.D. Muthiah, Global incidence and prevalence of nonalcoholic fatty liver disease, Clin. Mol. Hepatol. 29 (2023) S32.
[15] Z.M. Younossi, P. Golabi, J.M. Paik, A. Henry, C. Van Dongen, L. Henry, The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review, Hepatology 77 (2023) 1335.
[16] V.W.-S. Wong, M. Ekstedt, G.L.-H. Wong, H. Hagström, Changing epidemiology, global trends and implications for outcomes of NAFLD, J. Hepatol. 79 (3) (2023) 842–852.
[17] S. Murag, A. Ahmed, D. Kim, Recent epidemiology of nonalcoholic fatty liver disease, Gut Liver 15 (2021) 206.
[18] B.J. Perumpail, M.A. Khan, E.R. Yoo, G. Cholankeril, D. Kim, A. Ahmed, Clinical epidemiology and disease burden of nonalcoholic fatty liver disease, World J. Gastroenterol. 23 (2017) 8263.
[19] M. Alexander, A.K. Loomis, J. Fairburn-Beech, J. van der Lei, T. Duarte-Salles, D. Prieto-Alhambra, D. Ansell, A. Pasqua, F. Lapi, P. Rijnbeek, Real-world data reveal a diagnostic gap in non-alcoholic fatty liver disease, BMC Med. 16 (2018) 1–11.
[20] R. Loomba, L.A. Adams, The 20% rule of NASH progression: the natural history of advanced fibrosis and cirrhosis caused by NASH, Hepatology 70 (2019) 1885.
[21] S. Stender, R. Loomba, PNPLA3 genotype and risk of liver and all-cause mortality, Hepatology 71 (2020) 777.
[22] S. Singh, A.M. Allen, Z. Wang, L.J. Prokop, M.H. Murad, R. Loomba, Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies, Clin. Gastroenterol. Hepatol. 13 (2015) 643–654, https://doi.org/10.1016/j.cgh.2014.04.014.
[23] L.A. Adams, J.F. Lymp, J.S. Sauver, S.O. Sanderson, K.D. Lindor, A. Feldstein, P. Angulo, The natural history of nonalcoholic fatty liver disease: a population-based cohort study, Gastroenterology 129 (2005) 113–121.
[24] H. Yoshitaka, M. Hamaguchi, T. Kojima, T. Fukuda, A. Ohbora, M. Fukui, Nonoverweight nonalcoholic fatty liver disease and incident cardiovascular disease: a post hoc analysis of a cohort study, Medicine (Baltim.) 96 (2017).
[25] Z. Younossi, F. Tacke, M. Arrese, B. Chander Sharma, I. Mostafa, E. Bugianesi, V. Wai-Sun Wong, Y. Yilmaz, J. George, J. Fan, Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, Hepatology 69 (2019) 2672–2682.
[26] M.E. Rinella, J.V. Lazarus, V. Ratziu, S.M. Francque, A.J. Sanyal, F. Kanwal, D. Romero, M.F. Abdelmalek, Q.M. Anstee, J.P. Arab, A multi-society Delphi consensus statement on new fatty liver disease nomenclature, Ann. Hepatol. (2023) 101133.
[27] M.H. Wehmeyer, B.-C. Zyriax, B. Jagemann, E. Roth, E. Windler, J.S. Zur Wiesch, A.W. Lohse, J. Kluwe, Nonalcoholic fatty liver disease is associated with excessive calorie intake rather than a distinctive dietary pattern, Medicine (Baltim.) 95 (2016).
[28] M.A. Herman, V.T. Samuel, The sweet path to metabolic demise: fructose and lipid synthesis, Trends Endocrinol, Metab. 27 (2016) 719–730.
[29] M.C. Petersen, G.I. Shulman, Mechanisms of insulin action and insulin resistance, Physiol. Rev. 98 (2018) 2133–2223.
[30] J.M. Irimia, C.M. Meyer, D.M. Segvich, S. Surendran, A.A. DePaoli-Roach, N. Morral, P.J. Roach, Lack of liver glycogen causes hepatic insulin resistance and steatosis in mice, J. Biol. Chem. 292 (2017) 10455–10464.
[31] L.A. Lotta, P. Gulati, F.R. Day, F. Payne, H. Ongen, M. Van De Bunt, K.J. Gaulton, J.D. Eicher, S.J. Sharp, J. Luan, Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance, Nat. Genet. 49 (2017) 17–26.

[32] J.P. Camporez, Y. Wang, K. Faarkrog, N. Chukijrungroat, K.F. Petersen, G.I. Shulman, Mechanism by which arylamine N-acetyltransferase 1 ablation causes insulin resistance in mice, Proc. Natl. Acad. Sci. 114 (2017) E11285–E11292.
[33] D.J. Cuthbertson, T. Steele, J.P. Wilding, J.C. Halford, J.A. Harrold, M. Hamer, F. Karpe, What have human experimental overfeeding studies taught us about adipose tissue expansion and susceptibility to obesity and metabolic complications? Int. J. Obes. 41 (2017) 853–865.
[34] D.A. Guertin, D.M. Sabatini, Defining the role of mTOR in cancer, Cancer Cell 12 (2007) 9–22.
[35] Y. Nozaki, M.C. Petersen, D. Zhang, D.F. Vatner, R.J. Perry, A. Abulizi, S. Haedersdal, X.-M. Zhang, G.M. Butrico, V.T. Samuel, Metabolic control analysis of hepatic glycogen synthesis in vivo, Proc. Natl. Acad. Sci. 117 (2020) 8166–8176.
[36] R.J. Perry, X.-M. Zhang, D. Zhang, N. Kumashiro, J.-P.G. Camporez, G.W. Cline, D.L. Rothman, G.I. Shulman, Leptin reverses diabetes by suppression of the hypothalamic-pituitary-adrenal axis, Nat. Med. 20 (2014) 759–763.
[37] R.J. Perry, J.-P.G. Camporez, R. Kursawe, P.M. Titchenell, D. Zhang, C.J. Perry, M.J. Jurczak, A. Abudukadier, M.S. Han, X.-M. Zhang, Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes, Cell 160 (2015) 745–758.
[38] S. Li, M.S. Brown, J.L. Goldstein, Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis, Proc. Natl. Acad. Sci. 107 (2010) 3441–3446.
[39] K.F. Petersen, S. Dufour, A. Hariri, C. Nelson-Williams, J.N. Foo, X.-M. Zhang, J. Dziura, R.P. Lifton, G.I. Shulman, Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease, N. Engl. J. Med. 362 (2010) 1082–1089.
[40] D.F. Vatner, S.K. Majumdar, N. Kumashiro, M.C. Petersen, Y. Rahimi, A.K. Gattu, M. Bears, J.-P.G. Camporez, G.W. Cline, M.J. Jurczak, Insulin-independent regulation of hepatic triglyceride synthesis by fatty acids, Proc. Natl. Acad. Sci. 112 (2015) 1143–1148.
[41] K.W. Ter Horst, D.F. Vatner, D. Zhang, G.W. Cline, M.T. Ackermans, A.J. Nederveen, J. Verheij, A. Demirkiran, B.A. van Wagensveld, G.M. Dallinga-Thie, Hepatic insulin resistance is not pathway selective in humans with nonalcoholic fatty liver disease, Diabetes Care 44 (2021) 489–498.
[42] Y.G. Jeon, Y.Y. Kim, G. Lee, J.B. Kim, Physiological and pathological roles of lipogenesis, Nat. Metab. (2023) 1–25.
[43] Y.Y. Kim, H. Jang, G. Lee, Y.G. Jeon, J.H. Sohn, J.S. Han, W.T. Lee, J. Park, J.Y. Huh, H. Nahmgoong, Hepatic GSK3β-dependent CRY1 degradation contributes to diabetic hyperglycemia, Diabetes 71 (2022) 1373–1387.
[44] A. Caron, D. Richard, M. Laplante, The roles of mTOR complexes in lipid metabolism, Annu. Rev. Nutr. 35 (2015) 321–348.
[45] J.R. Cook, M.A. Hawkins, U.B. Pajvani, Liver insulinization as a driver of triglyceride dysmetabolism, Nat. Metab. 5 (2023) 1101–1110.
[46] S. Sookoian, C.J. Pirola, Genetics of nonalcoholic fatty liver disease: from pathogenesis to therapeutics, in: Semin. Liver Dis., Thieme Medical Publishers, 2019, pp. 124–140.
[47] C. Caussy, A. Tripathi, G. Humphrey, S. Bassirian, S. Singh, C. Faulkner, R. Bettencourt, E. Rizo, L. Richards, Z.Z. Xu, A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease, Nat. Commun. 10 (2019) 1–9.
[48] D. Stols-Gonçalves, L.S. Tristão, P. Henneman, M. Nieuwdorp, Epigenetic markers and microbiota/metabolite-induced epigenetic modifications in the pathogenesis of obesity, metabolic syndrome, type 2 diabetes, and non-alcoholic fatty liver disease, Curr. Diab. Rep. 19 (2019) 1–9.
[49] A. Zarrinpar, S. Gupta, M.R. Maurya, S. Subramaniam, R. Loomba, Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective study, Gut 65 (2016) 1546–1554.
[50] Q. Fang, Q. Shen, J. Li, Y. Yang, J. Guo, Y. Cheng, H. Zhou, W. Niu, L. Chen, C. Li, Inhibition of microRNA-124a attenuates non-alcoholic fatty liver disease through upregulation of adipose triglyceride lipase and the effect of liraglutide intervention, Hepatol. Res. 49 (2019) 743–757.
[51] S.R. Wesolowski, K.C. El Kasmi, K.R. Jonscher, J.E. Friedman, Developmental origins of NAFLD: a womb with a clue, Nat. Rev. Gastroenterol. Hepatol. 14 (2017) 81–96.
[52] K.J. Dudley, D.M. Sloboda, K.L. Connor, J. Beltrand, M.H. Vickers, 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 (2011) e21662.
[53] T.G. Oh, S.M. Kim, C. Caussy, T. Fu, J. Guo, S. Bassirian, S. Singh, E.V. Madamba, R. Bettencourt, L. Richards, A universal gut-microbiome-derived signature predicts cirrhosis, Cell Metab. 32 (2020) 878–888.
[54] L. Hoyles, J.-M. Fernandez-Real, M. Federici, M. Serino, J. Abbott, J. Charpentier, C. Heymes, J.L. Luque, E. Anthony, R.H. Barton, Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women, Nat. Med. 24 (2018) 1070–1080.
[55] J. Boursier, O. Mueller, M. Barret, M. Machado, L. Fizanne, F. Araujo-Perez, C.D. Guy, P.C. Seed, J.F. Rawls, L.A. David, The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota, Hepatology 63 (2016) 764–775.
[56] A. Peloso, M. Tihy, B. Moeckli, L. Rubbia-Brandt, C. Toso, Clearing steatosis prior to liver surgery for colorectal metastasis: a narrative review and case illustration, Nutrients 14 (2022) 5340.
[57] R.D. Welch, C. Billon, M. Losby, G. Bedia-Diaz, Y. Fang, A. Avdagic, B. Elgendy, T.P. Burris, K. Griffett, Emerging role of nuclear receptors for the treatment of NAFLD and NASH, Metabolites 12 (2022) 238.
[58] J.P. Arab, S.J. Karpen, P.A. Dawson, M. Arrese, M. Trauner, Bile acids and nonalcoholic fatty liver disease: molecular insights and therapeutic perspectives, Hepatology 65 (2017) 350–362.
[59] A. Bertolini, R. Fiorotto, M. Strazzabosco, Bile acids and their receptors: modulators and therapeutic targets in liver inflammation, in: Semin. Immunopathol., Springer, 2022, pp. 547–564.
[60] C.D. Fuchs, M. Trauner, Role of bile acids and their receptors in gastrointestinal and hepatic pathophysiology, Nat. Rev. Gastroenterol. Hepatol. 19 (2022) 432–450.
[61] O. Chávez-Talavera, A. Tailleux, P. Lefebvre, B. Staels, Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease, Gastroenterology 152 (2017) 1679–1694.
[62] S. McIlvride, V. Nikolova, H.M. Fan, J.A.K. McDonald, A. Wahlström, E. Bellafante, E. Jansen, L. Adorini, D. Shapiro, P. Jones, Obeticholic acid ameliorates dyslipidemia but not glucose tolerance in mouse model of gestational diabetes, Am. J. Physiol. Metab. 317 (2019) E399–E410.
[63] J. Chen, M. Thomsen, L. Vitetta, Interaction of gut microbiota with dysregulation of bile acids in the pathogenesis of nonalcoholic fatty liver disease and potential therapeutic implications of probiotics, J. Cell. Biochem. 120 (2019) 2713–2720.
[64] Y. Xiao, M. Kim, M.A. Lazar, Nuclear receptors and transcriptional regulation in non-alcoholic fatty liver disease, Mol. Metab. 50 (2021) 101119.
[65] Z. Moayedfard, F. Sani, A. Alizadeh, K. Bagheri Lankarani, M. Zarei, N. Azarpira, The role of the immune system in the pathogenesis of NAFLD and potential therapeutic impacts of mesenchymal stem cell-derived extracellular vesicles, Stem Cell Res. Ther. 13 (2022) 1–16.
[66] A.-M. Mirea, C.J. Tack, T. Chavakis, L.A.B. Joosten, E.J.M. Toonen, IL-1 family cytokine pathways underlying NAFLD: towards new treatment strategies, Trends Mol. Med. 24 (2018) 458–471.
[67] A.R. Mridha, A. Wree, A.A.B. Robertson, M.M. Yeh, C.D. Johnson, D.M. Van Rooyen, F. Haczeyni, N.C.-H. Teoh, C. Savard, G.N. Ioannou, NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice, J. Hepatol. 66 (2017) 1037–1046.
[68] B. Xu, M. Jiang, Y. Chu, W. Wang, D. Chen, X. Li, Z. Zhang, D. Zhang, D. Fan, Y. Nie, Gasdermin D plays a key role as a pyroptosis executor of non-alcoholic steatohepatitis in humans and mice, J. Hepatol. 68 (2018) 773–782.
[69] O. Krenkel, F. Tacke, Liver macrophages in tissue homeostasis and disease, Nat. Rev. Immunol. 17 (2017) 306–321.
[70] X. Xiong, H. Kuang, S. Ansari, T. Liu, J. Gong, S. Wang, X.-Y. Zhao, Y. Ji, C. Li, L. Guo, Landscape of intercellular crosstalk in healthy and NASH liver revealed by single-cell secretome gene analysis, Mol. Cell. 75 (2019) 644–660.
[71] M. Rau, A.-K. Schilling, J. Meertens, I. Hering, J. Weiss, C. Jurowich, T. Kudlich, H.M. Hermanns, H. Bantel, N. Beyersdorf, Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver, J. Immunol. 196 (2016) 97–105.
[72] G. Musso, R. Gambino, M. Cassader, E. Paschetta, A. Sircana, Specialized proresolving mediators: enhancing nonalcoholic steatohepatitis and fibrosis resolution, Trends Pharmacol. Sci. 39 (2018) 387–401.
[73] S. Schuster, D. Cabrera, M. Arrese, A.E. Feldstein, Triggering and resolution of inflammation in NASH, Nat. Rev. Gastroenterol. Hepatol. 15 (2018) 349–364.
[74] S. Hwang, H. Yun, S. Moon, Y.E. Cho, B. Gao, Role of neutrophils in the pathogenesis of nonalcoholic steatohepatitis, Front. Endocrinol. 12 (2021) 751802.

[75] B. González-Terán, N. Matesanz, I. Nikolic, M.A. Verdugo, V. Sreeramkumar, L. Hernández-Cosido, A. Mora, G. Crainiciuc, M.L. Sáiz, E. Bernardo, p38γ and p38δ reprogram liver metabolism by modulating neutrophil infiltration, EMBO J. 35 (2016) 536–552.
[76] C.J. Calvente, M. Tameda, C.D. Johnson, H. Del Pilar, Y.C. Lin, N. Adronikou, X.D.M. Du Jeu, C. Llorente, J. Boyer, A.E. Feldstein, Neutrophils contribute to spontaneous resolution of liver inflammation and fibrosis via microRNA-223, J. Clin. Invest. 129 (2019) 4091–4109.
[77] T. Hendrikx, F. Porsch, M.G. Kiss, D. Rajcic, N. Papac-Miličević, C. Hoebinger, L. Goederle, A. Hladik, L.E. Shaw, H. Horstmann, Soluble TREM2 levels reflect the recruitment and expansion of TREM2+ macrophages that localize to fibrotic areas and limit NASH, J. Hepatol. 77 (2022) 1373–1385.
[78] I. Liebold, S. Meyer, M. Heine, A. Kuhl, J. Witt, L. Eissing, A.W. Fischer, A.C. Koop, J. Kluwe, J. Schulze zur Wiesch, TREM2 regulates the removal of apoptotic cells and inflammatory processes during the progression of NAFLD, Cells 12 (2023) 341.
[79] J. Hou, J. Zhang, P. Cui, Y. Zhou, C. Liu, X. Wu, Y. Ji, S. Wang, B. Cheng, H. Ye, TREM2 sustains macrophage-hepatocyte metabolic coordination in nonalcoholic fatty liver disease and sepsis, J. Clin. Invest. 131 (2021).
[80] S. Sutti, E. Albano, Adaptive immunity: an emerging player in the progression of NAFLD, Nat. Rev. Gastroenterol. Hepatol. 17 (2020) 81–92.
[81] F. Barrow, S. Khan, H. Wang, X.S. Revelo, The emerging role of B cells in the pathogenesis of NAFLD, Hepatology 74 (2021) 2277–2286.
[82] C.-J. Deng, T.-H. Lo, K.-Y. Chan, X. Li, M.-Y. Wu, Z. Xiang, C.-M. Wong, Role of B lymphocytes in the pathogenesis of NAFLD: a 2022 update, Int. J. Mol. Sci. 23 (2022) 12376.
[83] L. Petagine, M.G. Zariwala, V.B. Patel, Non-alcoholic fatty liver disease: immunological mechanisms and current treatments, World J. Gastroenterol. 29 (2023) 4831.
[84] P. Hirsova, A.O. Bamidele, H. Wang, D. Povero, X.S. Revelo, Emerging roles of T cells in the pathogenesis of nonalcoholic steatohepatitis and hepatocellular carcinoma, Front. Endocrinol. 12 (2021) 760860.
[85] M. Dudek, D. Pfister, S. Donakonda, P. Filpe, A. Schneider, M. Laschinger, D. Hartmann, N. Hueser, P. Meiser, F. Bayerl, Auto-aggressive CXCR6+ CD8 T cells cause liver immune pathology in NASH, Nature 592 (2021) 444–449.
[86] Y. Li, Z. You, R. Tang, X. Ma, Tissue-resident memory T cells in chronic liver diseases: phenotype, development and function, Front. Immunol. 13 (2022) 967055.
[87] P. An, L.-L. Wei, S. Zhao, D.Y. Sverdlov, K.A. Vaid, M. Miyamoto, K. Kuramitsu, M. Lai, Y.V. Popov, Hepatocyte mitochondria-derived danger signals directly activate hepatic stellate cells and drive progression of liver fibrosis, Nat. Commun. 11 (2020) 1–15.
[88] M. Grohmann, F. Wiede, G.T. Dodd, E.N. Gurzov, G.J. Ooi, T. Butt, A.A. Rasmiena, S. Kaur, T. Gulati, P.K. Goh, Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC, Cell. 175 (2018) 1289–1306.
[89] M. Du, X. Wang, L. Yuan, B. Liu, X. Mao, D. Huang, L. Yang, K. Huang, F. Zhang, Y. Wang, Targeting NFATc4 attenuates non-alcoholic steatohepatitis in mice, J. Hepatol. 73 (2020) 1333–1346.
[90] Z. Song, W. Chen, D. Athavale, X. Ge, R. Desert, S. Das, H. Han, N. Nieto, Osteopontin takes center stage in chronic liver disease, Hepatology 73 (2021) 1594–1608.
[91] Z. Du, Z. Lin, Z. Wang, D. Liu, D. Tian, L. Xia, SPOCK1 overexpression induced by platelet-derived growth factor-BB promotes hepatic stellate cell activation and liver fibrosis through the integrin α5β1/PI3K/Akt signaling pathway, Lab. Investig. 100 (2020) 1042–1056.
[92] D.S. Ghorpade, L. Ozcan, Z. Zheng, S.M. Nicoloro, Y. Shen, E. Chen, M. Blüher, M.P. Czech, I. Tabas, Hepatocyte-secreted DPP4 in obesity promotes adipose inflammation and insulin resistance, Nature 555 (2018) 673–677.
[93] E. Somm, H. Henry, S.J. Bruce, S. Aeby, M. Rosikiewicz, G.P. Sykiotis, M. Asrih, F.R. Jornayvaz, P.D. Denechaud, U. Albrecht, β-Klotho deficiency protects against obesity through a crosstalk between liver, microbiota, and brown adipose tissue, JCI Insight 2 (2017).
[94] X. Brenachot, G. Ramadori, R.M. Ioris, C. Veyrat-Durebex, J. Altirriba, E. Aras, S. Ljubicic, D. Kohno, S. Fabbiano, S. Clement, Hepatic protein tyrosine phosphatase receptor gamma links obesity-induced inflammation to insulin resistance, Nat. Commun. 8 (2017) 1–9.
[95] L. Guo, P. Zhang, Z. Chen, H. Xia, S. Li, Y. Zhang, S. Kobberup, W. Zou, J.D. Lin, Hepatic neuregulin 4 signaling defines an endocrine checkpoint for steatosis-to-NASH progression, J. Clin. Invest. 127 (2017) 4449–4461.
[96] N. Liang, A. Damdimopoulos, S. Goñi, Z. Huang, L.-L. Vedin, T. Jakobsson, M. Giudici, O. Ahmed, M. Pedrelli, S. Barilla, Hepatocyte-specific loss of GPS2 in mice reduces non-alcoholic steatohepatitis via activation of PPARα, Nat. Commun. 10 (2019) 1–14.
[97] A. Bayoumi, H. Grønbæk, J. George, M. Eslam, The epigenetic drug discovery landscape for metabolic-associated fatty liver disease, Trends Genet. 36 (2020) 429–441.
[98] S. Wang, L. Zha, X. Cui, Y. Yeh, R. Liu, J. Jing, H. Shi, W. Chen, J. Hanover, J. Yin, Epigenetic regulation of hepatic lipid metabolism by DNA methylation, Adv. Sci. (2023) 2206068.
[99] A. Hussain Nasrullah, S. Ahmed, M. Rasool, A.J. Shah, DNA methylation across the tree of life, from micro to macro-organism, Bioengineered 13 (2022) 1666–1685.
[100] W. Chang, Y. Zhao, D. Rayée, Q. Xie, M. Suzuki, D. Zheng, A. Cvekl, Dynamic changes in whole genome DNA methylation, chromatin and gene expression during mouse lens differentiation, Epigenet. Chromatin 16 (2023) 4.
[101] A. Morishita, K. Oura, T. Tadokoro, K. Fujita, J. Tani, H. Kobara, M. Ono, T. Himoto, T. Masaki, MicroRNAs and nonalcoholic steatohepatitis: a review, Int. J. Mol. Sci. 24 (2023) 14482.
[102] S.H. Ibrahim, P. Hirsova, G.J. Gores, Non-alcoholic steatohepatitis pathogenesis: sublethal hepatocyte injury as a driver of liver inflammation, Gut 67 (2018) 963–972.
[103] R.F. Schwabe, T. Luedde, Apoptosis and necroptosis in the liver: a matter of life and death, Nat. Rev. Gastroenterol. Hepatol. 15 (2018) 738–752.
[104] P.-X. Wang, Y.-X. Ji, X.-J. Zhang, L.-P. Zhao, Z.-Z. Yan, P. Zhang, L.-J. Shen, X. Yang, J. Fang, S. Tian, Targeting CASP8 and FADD-like apoptosis regulator ameliorates nonalcoholic steatohepatitis in mice and nonhuman primates, Nat. Med. 23 (2017) 439–449.
[105] P. Zhang, P.-X. Wang, L.-P. Zhao, X. Zhang, Y.-X. Ji, X.-J. Zhang, C. Fang, Y.-X. Lu, X. Yang, M.-M. Gao, The deubiquitinating enzyme TNFAIP3 mediates inactivation of hepatic ASK1 and ameliorates nonalcoholic steatohepatitis, Nat. Med. 24 (2018) 84–94.
[106] P. Hirsova, P. Weng, W. Salim, S.F. Bronk, T.S. Griffith, S.H. Ibrahim, G.J. Gores, TRAIL deletion prevents liver inflammation but not adipose tissue inflammation during murine diet-induced obesity, Hepatol. Commun. 1 (2017) 648–662.
[107] J.Y. Kim, R. Garcia-Carbonell, S. Yamachika, P. Zhao, D. Dhar, R. Loomba, R.J. Kaufman, A.R. Saltiel, M. Karin, ER stress drives lipogenesis and steatohepatitis via caspase-2 activation of S1P, Cell. 175 (2018) 133–145.
[108] F. Negro, Natural history of NASH and HCC, Liver Int. 40 (2020) 72–76.
[109] S. Mittal, H.B. El-Serag, Y.H. Sada, F. Kanwal, Z. Duan, S. Temple, S.B. May, J.R. Kramer, P.A. Richardson, J.A. Davila, Hepatocellular carcinoma in the absence of cirrhosis in United States veterans is associated with nonalcoholic fatty liver disease, Clin. Gastroenterol. Hepatol. 14 (2016) 124–131.
[110] F. Piscaglia, G. Svegliati-Baroni, A. Barchetti, A. Pecorelli, S. Marinelli, C. Tiribelli, S. Bellentani, H.I.S. Group, Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study, Hepatology 63 (2016) 827–838.
[111] A. Berkan-Kawińska, A. Piekarska, Hepatocellular carcinoma in non-alcohol fatty liver disease–changing trends and specific challenges, Curr. Med. Res. Opin. 36 (2020) 235–243.
[112] N.P. Chalasani, T.S. Ramasubramanian, A. Bhattacharya, M.C. Olson, L.R. Roberts, J.B. Kisiel, K.R. Reddy, G.P. Lidgard, S.C. Johnson, J.J. Bruinsma, A novel blood-based panel of methylated DNA and protein markers for detection of early-stage hepatocellular carcinoma, Clin. Gastroenterol. Hepatol. 19 (2021) 2597–2605.
[113] K. Martin, A. Hatab, V.S. Athwal, E. Jokl, K. Piper Hanley, Genetic contribution to non-alcoholic fatty liver disease and prognostic implications, Curr. Diab. Rep. 21 (2021) 1–8.
[114] E. Paolini, M. Longo, M. Meroni, G. Tria, A. Cespiati, R. Lombardi, S. Badiali, M. Maggioni, A.L. Fracanzani, P. Dongiovanni, The I148M PNPLA3 variant mitigates niacin beneficial effects: how the genetic screening in non-alcoholic fatty liver disease patients gains value, Front. Nutr. 10 (2023) 1101341.
[115] K. Bhaskaran, I. Douglas, H. Forbes, I. dos-Santos-Silva, D.A. Leon, L. Smeeth, Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5· 24 million UK adults, Lancet. 384 (2014) 755–765.

[116] I. Doycheva, T. Zhang, W. Amjad, P.J. Thuluvath, Diabetes and hepatocellular carcinoma: incidence trends and impact of liver disease etiology, J. Clin. Exp. Hepatol. 10 (2020) 296–303.
[117] J.D. Yang, F. Ahmed, K.C. Mara, B.D. Addissie, A.M. Allen, G.J. Gores, L.R. Roberts, Diabetes is associated with increased risk of hepatocellular carcinoma in patients with cirrhosis from nonalcoholic fatty liver disease, Hepatology 71 (2020) 907–916.
[118] M. Masarone, V. Rosato, M. Dallio, A.G. Gravina, A. Aglitti, C. Loguercio, A. Federico, M. Persico, Role of oxidative stress in pathophysiology of nonalcoholic fatty liver disease, Oxid. Med. Cell. Longev. 2018 (2018).
[119] Y. Li, L. Li, D. Fan, Z. Wang, Y. Cui, Effects of GST null genotypes on individual susceptibility to atherosclerotic cardiovascular diseases: a meta-analysis, Free Radic. Res. 54 (2020) 567–573.
[120] J.Q. Liang, N. Teoh, L. Xu, S. Pok, X. Li, E.S.H. Chu, J. Chiu, L. Dong, E. Arfianti, W.G. Haigh, Dietary cholesterol promotes steatohepatitis related hepatocellular carcinoma through dysregulated metabolism and calcium signaling, Nat. Commun. 9 (2018) 1–13.
[121] D. Liu, C.C. Wong, L. Fu, H. Chen, L. Zhao, C. Li, Y. Zhou, Y. Zhang, W. Xu, Y. Yang, Squalene epoxidase drives NAFLD-induced hepatocellular carcinoma and is a pharmaceutical target, Sci. Transl. Med. 10 (2018) eaap9840.
[122] E. Piccinin, G. Villani, A. Moschetta, Metabolic aspects in NAFLD, NASH and hepatocellular carcinoma: the role of PGC1 coactivators, Nat. Rev. Gastroenterol. Hepatol. 16 (2019) 160–174.
[123] D. Suresh, A.N. Srinivas, A. Prashant, S. Satish, P. Vishwanath, S.M. Nataraj, S.V. Koduru, P.K. Santhekadur, D.P. Kumar, AATF inhibition exerts antiangiogenic effects against human hepatocellular carcinoma, Front. Oncol. 13 (2023) 1130380.
[124] P. Hirsova, F. Bohm, E. Dohnalkova, B. Nozickova, M. Heikenwalder, G.J. Gores, A. Weber, Hepatocyte apoptosis is tumor promoting in murine nonalcoholic steatohepatitis, Cell Death Dis. 11 (2020) 1–12.
[125] Y.A. Lee, L.A. Noon, K.M. Akat, M.D. Ybanez, T.-F. Lee, M.-L. Berres, N. Fujiwara, N. Goossens, H.-I. Chou, F.P. Parvin-Nejad, Autophagy is a gatekeeper of hepatic differentiation and carcinogenesis by controlling the degradation of Yap, Nat. Commun. 9 (2018) 1–12.
[126] M. Maurel, A. Samali, E. Chevet, Endoplasmic reticulum stress: at the crossroads of inflammation and metabolism in hepatocellular carcinoma development, Cancer Cell 26 (2014) 301–303.
[127] D. Xu, M. Xu, S. Jeong, Y. Qian, H. Wu, Q. Xia, X. Kong, The role of Nrf2 in liver disease: novel molecular mechanisms and therapeutic approaches, Front. Pharmacol. 9 (2019) 1428.
[128] Z. Chen, C. Ding, Y. Gu, Y. He, B. Chen, S. Zheng, Q. Li, Association between gut microbiota and hepatocellular carcinoma from 2011 to 2022: bibliometric analysis and global trends, Front. Oncol. 13 (2023) 1120515.
[129] T. Kobayashi, M. Iwaki, A. Nakajima, A. Nogami, M. Yoneda, Current research on the pathogenesis of NAFLD/NASH and the gut–liver Axis: gut microbiota, dysbiosis, and leaky-gut syndrome, Int. J. Mol. Sci. 23 (2022) 11689.
[130] S. Sydor, J. Best, I. Messerschmidt, P. Manka, R. Vilchez-Vargas, S. Brodesser, C. Lucas, A. Wegehaupt, C. Wenning, S. Aßmuth, Altered microbiota diversity and bile acid signaling in cirrhotic and noncirrhotic NASH-HCC, Clin. Transl. Gastroenterol. 11 (2020).
[131] H. Chu, B. Williams, B. Schnabl, Gut microbiota, fatty liver disease, and hepatocellular carcinoma, Liver Res 2 (2018) 43–51.
[132] S. Yoshimoto, T.M. Loo, K. Atarashi, H. Kanda, S. Sato, S. Oyadomari, Y. Iwakura, K. Oshima, H. Morita, M. Hattori, Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome, Nature 499 (2013) 97–101.
[133] C. Ma, M. Han, B. Heinrich, Q. Fu, Q. Zhang, M. Sandhu, D. Agdashian, M. Terabe, J.A. Berzofsky, V. Fako, Gut microbiome–mediated bile acid metabolism regulates liver cancer via NKT cells, Science 360 (2018) eaan5931.
[134] Z. Dong, Q. Zhuang, M. Ning, S. Wu, L. Lu, X. Wan, Palmitic acid stimulates NLRP3 inflammasome activation through TLR4-NF-κB signal pathway in hepatic stellate cells, Ann. Transl. Med. 8 (2020).
[135] R. Loomba, A.J. Sanyal, K.V. Kowdley, D.L. Bhatt, N. Alkhouri, J.P. Frias, P. Bedossa, S.A. Harrison, D. Lazas, R. Barish, Randomized, controlled trial of the FGF21 analogue pegozafermin in NASH, N. Engl. J. Med. 398 (11) (2023) 998–1008.
[136] R.R. Nathani, M.B. Bansal, Update on clinical trials for nonalcoholic steatohepatitis, Gastroenterol. Hepatol. 19 (2023) 371.
[137] S.A. Harrison, P. Bedossa, C.D. Guy, J.M. Schattenberg, R. Loomba, R. Taub, D. Labriola, S.E. Moussa, G.W. Neff, M.E. Rinella, A phase 3, randomized, controlled trial of resmetirom in NASH with liver fibrosis, N. Engl. J. Med. 390 (2024) 497–509.
[138] L. Rong, J. Zou, W. Ran, X. Qi, Y. Chen, H. Cui, J. Guo, Advancements in the treatment of non-alcoholic fatty liver disease (NAFLD), Front. Endocrinol. 13 (2023) 1087260.
[139] N.G.C. Uk, Non-Alcoholic Fatty Liver Disease, Assessment and Management, 2016.
[140] V. Ratziu, M. Hompesch, M. Petitjean, C. Serdjebi, J.S. Iyer, A.V. Parwani, D. Tai, E. Bugianesi, K. Cusi, S.L. Friedman, Artificial intelligence-assisted digital pathology for non-alcoholic steatohepatitis: current status and future directions, J. Hepatol. 80 (2) (2024) 335–351.
[141] M. Martic, D. Tai, N. Naoumov, E. Chng, C. Brass, J. Loeffler, J.G. Kochuparampil, J.L. Chong, S. Ma, A. Sailer, Magnetic resonance elastography (MRE) demonstrate the strongest correlation with digital pathology and NASH CRN fibrosis assessments, compared to transient elastography and other assessed non-invasive tests (NITs), J. Hepatol. 77 (2022) S493.