Chapter

The Rise in the Prevalence of Nonalcoholic Fatty Liver Disease and Hepatocellular Carcinoma

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Abstract

Nonalcoholic fatty liver disease (NAFLD) affects a third of the world's population and its rapid rise parallels the increase in hepatocellular carcinoma (HCC). NAFLD replacing hepatitis C virus (HCV) infection as a leading indicator for liver transplantation (LT) in the United States. NAFLD is a spectrum of disease ranging from simple steatosis (NAFL) to nonalcoholic steatohepatitis (NASH), which can progress to advanced fibrosis (AF) and cirrhosis, culminating in HCC. The main clinical concern of public health administrators is that many patients who are unaware of NAFLD remain undiagnosed and risk developing end-stage liver disease (ESLD). Clinicians overly rely on surrogate liver enzymes to identify patients with NAFLD, allowing for substantial liver disease to go unnoticed and untreated. Furthermore, according to epidemiological studies, in patients diagnosed with NAFLD, ethnicity plays a role in complications and treatment response, and ethnic correlations with NAFLD are thoroughly underreported. Although liver biopsy is the gold standard method for appropriately diagnosing and staging NAFLD, most patients can be effectively diagnosed non-invasively with imaging modalities and integrated tests that are routinely available in the clinic today. This chapter discusses the current global rise in the rates of NAFLD and HCC; the current key findings incidences and the recommended diagnostic approaches and in therapeutic methods.

Keywords: obesity, cirrhosis, hepatocellular carcinoma (HCC), insulin resistance (IR), liver transplantation (LT), metabolic syndrome (MetS), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH)

1. Introduction

The liver is a 1.5 kg reddish-brown biochemical processing plant of immense responsibilities that include protein synthesis, xenobiotic or drug metabolism, blood detoxification, and the release of bile acids for digestion. In short, the liver plays a key role in the hemostasis of the body by regulating the levels of sugar, protein, and fat that circulate in the blood. However, obesity, which must be carefully defined according to ethnic-specific BMI cut-off points, may alter normal liver physiology and lead to liver disease [1]. Obesity is at the intersection of the chronic liver disease pathway that includes diabetes, metabolic syndrome (MetS), nonalcoholic fatty liver disease (NAFLD), and hepatocellular carcinoma (HCC). The complex association between obesity and liver function involving NAFLD, HCC, histopathology, and genetic factors is the subject of several collaborative research investigations [2–7].

Over the past few decades, dramatic changes in lifestyle behaviors and health priorities have contributed to a significant rise in noncommunicable diseases such as obesity and NAFLD. Obesity is highly prevalent in the United States of America, estimated to represent between 30 and 38% of adults with a body mass index (BMI) greater than 30 kg/m^2 [8, 9]. Obesity is also a risk factor for metabolic syndrome (MetS), which increases hepatic triglyceride (TGs) depositions. NAFLD is the most common cause of impaired liver function in Western countries, affecting over one quarter of the population [10, 11]. Obesity is driving the rise of NAFLD and nonalcoholic steatohepatitis (NASH), the culmination of the fatty liver disease spectrum that is manifested by ballooning, scarring, cirrhosis, and finally liver failure and HCC [12]. It is estimated that globally the prevalence of NAFLD in the general population is 24–30% [13, 14]. Accounting for errors in accuracy that may exist in indirect measurement methodologies, in the United States, the prevalence of NAFLD in adults has risen from 18% in 1988–1991, to 29% in 1999–2000, to 31% in 2011–2012 [15]. However, the prevalence of NAFLD in the United States diagnosed by ultrasonography alone was estimated to be 24.13% (95% CI 19.73–29.15%) [16].

1.1 NAFLD definition

Nonalcoholic fatty liver disease (NAFLD) is a broad-spectrum disease ranging from fat infiltration of hepatocytes with no symptoms (simple steatosis aka nonalcoholic fatty liver, NAFL) to excess intrahepatic macroglobular and macrovesicular fat accumulation (5–10% by weight of liver) with aggravated inflammation (steatohepatitis aka nonalcoholic steatohepatitis, NASH) in the presence of little ethanol (typically <30 g per day for men and <20 g per day for women) or no alcohol consumption in the last 12 months [12, 17]. It should be noted, however, there is now convincing evidence demonstrating that even "safe" levels of alcohol consumption are associated with adverse health outcomes [17–20] suggesting that future studies should include only nondrinker individuals in the "NAFLD definition" [21]. Therefore, for NAFLD classification, the patient must show evidence of hepatic fat accumulation in the absence of declared chronic alcohol consumption, or drug use that can induce steatosis, or hereditary disorders. This NAFLD designation excludes both macrovesicular and microvesicular steatosis encompassing certain drugs, toxins, viral hepatitis B (HBV), hepatitis C (HCV) or human immunodeficiency virus (HIV) infections, celiac disease, α-1 antitrypsin deficiency, hepatobiliary infectious diseases, hepatolenticular degeneration, hepatic malignancies, genetic hemochromatosis, Wilson's disease, lipodystrophy, abetalipoproteinemia, Reye's syndrome, HELLP syndrome, or decompensated cirrhosis, which may contribute to secondary causes of steatosis and elevated liver enzymes [22–24]. Additional medications targeted for exclusion are estrogen, sodium valproate, nonsteroidal anti-inflammatory drugs (NSAIDs), calcium antagonists, perhexiline-maleate, and antiretroviral drugs [25–27]. Appropriate medical history must also be taken to exclude the uncommon causes of fatty liver secondary to treatment with drugs such as amiodarone, diltiazem, steroids, synthetic estrogens, tamoxifen, and highly active antiretroviral therapy; refeeding syndrome and total parenteral nutrition; severe weight loss after jejunoileal or gastric bypass; lipodystrophy; and other rare disorders [28]. There are also strong opinions for the exclusion of "whole-body system diseases" such as inflammatory bowel syndrome, hypothyroidism, and lipoatrophy [25] from the "secondary fatty liver diseases" category because they may also induce liver steatosis.

NAFLD can be distinguished from alcoholic steatohepatitis (ASH) by the absence of alcohol consumption and on histological markers such as sclerosing hyaline necrosis, hepatocyte ballooning, portal granulocytic inflammation, lobular

inflammation, satellitosis, perisinusoidal fibrosis, Mallory-Denk bodies, and acute cholestasis among others [29–31]. However, it is important to note that NAFLD can also coexist with other liver diseases including HCV, hemochromatosis, and alcoholic liver disease, which can accelerate progression to end-stage liver disease (ESLD) [32].

1.2 The natural history of NAFLD

The pathophysiology of NAFLD and its variants is still incompletely understood thereby limiting the availability of effective diagnostic and therapeutic intervention. The ongoing persistence of obesity and the accompanying high rates of diabetes will increase the prevalence of NAFLD [33]. In many cases, the natural cause of the disease is the development of cirrhosis and ESLD as the population ages. Increased mortality rates have been reported in studies that compared NAFLD patients with a normal reference population [34–36]. The primary cause of death for NAFLD patients is cardiovascular disease followed by nonliver cancer, whereas the third leading cause of mortality is liver-related complications including cirrhosis [33]. The exact prevalence of fatty liver condition is not known, but population studies from the United States and China estimate that 28–30% of the general population has simple steatosis that carries a relatively benign prognosis and is measured using magnetic resonance spectroscopy (the most accurate imaging modality) and that 8% of the population has elevated alanine transaminase (ALT) [37, 38]. A follow-up of population-based studies examining the natural history of NAFLD patients in Minnesota revealed that 3.1% of the patients developed cirrhosis-related complications including ascites (2%), jaundice (2%), encephalopathy (2%), variceal bleeding (1%), and HCC (0.5%) [34]. Approximately 10–30% of those with steatosis develop NASH, and the development of NASH cirrhosis is associated with a poor long-term prognosis for 2.6% of them who will be at a risk of developing HCC [39–41]. Ten years following diagnosis, 45% will decompensate and the mortality rate for subjects with Child-Pugh A disease will be 20% [42]. Furthermore, besides having an increased liver-related mortality rate compared to the general population, patients with NASH also have an increased risk of cardiovascular death (15.5 vs. 7.5%, $p = 0.04$) [35]. Generally, NAFLD is a slowly progressing disease, which does not culminate in ESLD in most patients. Identifying those who will develop a complete liver failure is a difficult proposition [43]. NAFLD data are limited on predictors of clinical progression to NASH and beyond. Due to the compounding effect of obesity, prospective longitudinal studies are needed to help in the prediction of outcomes for individual patients. On the other hand, patients with NASH have a worse prognosis and attempts should be made to include them in clinical trials of novel treatments for this condition. The sequence of steps in liver disease commencing with steatosis and eventually culminating in HCC (i.e., ESLD) is presented in **Figure 1** [44].

1.3 NAFLD diagnosis and staging

The general classification of NAFLD as stated above and accepted by the American Association for the Study of Liver Diseases (AASLD) is a hepatic fat accumulation exceeding 5–10% by weight of the liver [45]. Accordingly, NAFLD diagnosis in the liver is based on: (i) the presence of simple steatosis, as determined by histological or imaging procedure; (ii) a total weekly consumption of less than 140 g of ethanol for men and less than 70 g for women in the last 12 months; and (iii) the absence of competing etiologies for simple liver steatosis and the absence of coexisting causes for chronic liver disease [46]. An appropriate diagnosis of

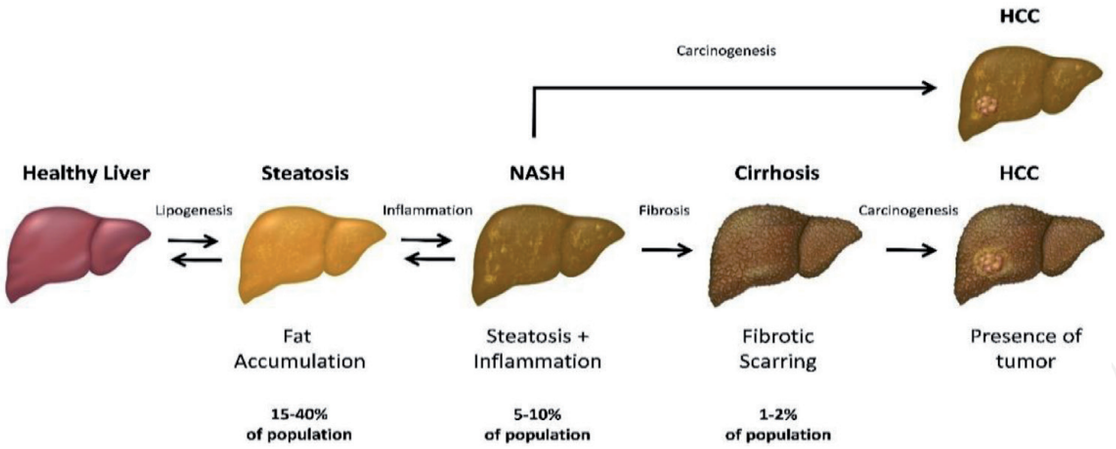

Figure 1.
The progression and stages of NAFLD (adapted from Baranova et al., [44]). Steatosis is the initial NAFLD stage and is characterized by excessive accumulation of fat in hepatocytes. Subsequent inflammatory conditions accelerate the progression to NASH followed by liver cirrhosis, which may lead to HCC. Both steatosis and NASH can reverse to NAFLD.

NAFLD, which is multifaceted, requires that there is evidence of hepatic steatosis upon imaging and histology or both and that other causes of liver disease including steatosis have been excluded [23].

The increasing prevalence of obesity in the past few decades has led to a surge in NAFLD, which manifests liver cells as bloated with droplets of fat. It has been reported that 70% of centrally obese patients with diabetes and hypertension (HTN) harbor steatohepatitis on liver biopsy [47]. Imaging has enabled the observation of central obesity in 70–80% of these subjects and in 50–80% of patients with type 2 diabetes mellitus (T2DM). NAFLD is typically asymptomatic; therefore, diagnosis usually follows the subsidiary finding of abnormal liver enzymes or steatosis on imaging. Early diagnosis of NAFLD requires skilled and informed practitioners to halt fibrosis progression to more advanced stages. Liver needle puncture biopsy, although invasive, is the gold standard. Less-invasive methods of image detection tools may not provide consistent information due to the subjective interpretations of the data by radiologists [48]. But imaging tools such as abdominal ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) are beginning to meet this need. Ultrasound or sonography is very effective in diagnosing steatosis where greater than 33% of hepatocytes are steatotic but can be unreliable with lesser degrees of steatosis [49]. The other imaging modalities such as CT or MRI can also detect hepatic steatosis even though they are not used in the evaluation of steatosis. Currently, the combination of MRI and proton magnetic resonance spectroscopy (MRI/^{1}H-MRS) is the most accurate noninvasive measuring tool of steatosis [50, 51]. ^{1}H-MRS, which defines NAFLD as hepatic fat accumulation (steatosis) >5% of total weight of the liver, is the most reliable quantitative tool. However, due to its prohibitive cost, it is not widely available. Ultrasonography, on the other hand, is the instrument of choice for most of the clinics due to its low cost and wide availability even though it is still relatively limited in the detection of inflammation, a more important and higher risk concern than steatosis for fibrosis, cirrhosis, and HCC [52, 53].

Controlled attenuation parameter (CAP), which is a novel ultrasound-based technique that assesses liver stiffness and steatosis simultaneously by employing transient elastography (TE) [54]. This CAP technique has been shown to accurately detect steatosis although its diagnostic threshold has not yet been determined. Obesity and diabetes are the main risk factors for NAFLD [55]. It has been reported that the presence of T2DM significantly increases the prevalence

of NAFLD regardless of the diagnostic tool [56]. For example, using controlled attenuation parameter (CAP), the prevalence of NAFLD is estimated at 75% in T2DM population and 40% in the general population, whereas it is 65% and about 37% respectively when measured by ^{1}H-MRS. The prevalence rate goes down when assessed by liver ultrasound, computed tomography, and plasma ALT in that order [56].

In contrast, most global population studies base their NAFLD characterization on less sensitive and less specific surrogate markers of the disease including elevated liver-associated enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT >40 IU/L in males; >31 IU/L in females) [57, 58]. Furthermore, serum ALT levels are within the range currently considered "normal" in a sizeable proportion of NAFLD subjects [59]. Typically, depending on the reference values from different laboratories, the broad range for normal AST is reported between 10 and 40 IU/L and ALT between 7 and 56 IU/L. This is because ALT usually falls (and AST may rise) as fibrosis progresses to cirrhosis. Mild elevations, which are generally asymptomatic, are considered to be 2–3 times higher than the normal range, and drastic elevations are 5 times higher than the upper limit of normal, which varies according to gender [60]. Moreover, the very selective measurement of ALT level based on race or ethnicity underscores the lack of effective surrogate markers for NAFLD/NASH in the absence of biopsy [61]. Therefore, an innovative approach is needed to use metabolic risk factors to identify subjects with NAFLD/NASH rather than relying on liver enzyme abnormalities.

There is an active research that is underway to discover serum biomarkers for NASH since it is associated with increased apoptosis and therefore blood markers of apoptosis may be instrumental in distinguishing NASH from simple steatosis [62]. Apoptosis activates caspases that cleave various substrates such as cytokeratin-18 (CK-18), a key intermediate filament protein in hepatocytes, that can be detected with an ELISA test using an M30 antibody to identify patients with NASH [63, 64]. However, liver biopsy provides a superior assessment of hepatic steatosis, hepatocellular injury, inflammation, and fibrosis as well as its ability to demonstrate the presence of hepatocyte ballooning and degeneration in association with steatosis as the key histological feature that distinguishes NASH from simple steatosis. Notwithstanding its limitations such as inherent variability in histologic assessment of NAFLD stage and activity, its invasiveness, its high possibility of complications related to liver damage, its proneness to sampling error generated by the operators, and its limitations in accessibility and reproducibility, liver biopsy is still the standard criterion for the most accurate diagnosis of NAFLD and NASH. Also, because only 7–30% of NAFLD patients in the world population had an indication of biopsy for accurate measurement, Younosis et al., re-evaluated and reported the global prevalence of NASH to be between 1.5 and 6.45% and the North American rate at an average of 8.69% (between 7.2 and 14.63%) [4, 65]. Regarding obesity, reports show that NASH can be verified by histological examination in about 47% of all NAFLD cases among obese individuals [66].

Liver fibrosis is the inordinate accretion of extracellular matrix proteins that include collagen in most types of liver disease including NAFLD. Fibrosis stage is a crucial histological variable to predict mortality. There are well-known independent predictors of fibrosis, which is a subway to chronic liver disease state. Some of these risk factors are age (>45–50), BMI (>28–30 kg/m^2), insulin resistance (IR), diabetes, and HTN [67]. Staging hepatic fibrosis is essential in all patients with NAFLD to identify individuals with advanced fibrosis (AF) who may later develop liver-related complications such as hepatocellular dysfunction and portal hypertension

(PHTN). A noninvasive and an indirect assessment, which is performed in all liver disease patients including children, may include blood tests such as liver function tests (low albumin), complete blood count (thrombocytopenia and neutropenia), and coagulation profile (prolonged prothrombin time) [68]. Among the diagnostic tools used to measure the prevalence of AF in the setting of T2DM versus the general population, vibration-controlled transient elastography shows the highest prevalence rate followed by NAFLD fibrosis score and FibroTest in that order. It should be noted that the prevalence of T2DM significantly increases the prevalence of AF in similar ways to NAFLD [56].

The most widespread clinically implemented histological grading and staging system is the 'NAFLD activity score' (NAS) [6] (see **Table 1**). More recently, the SAF score encompassing an assessment of steatosis (S), activity (A), and fibrosis (F) has been used to produce more accurate measurements of NASH [5]. These recent developments underscore the fact that NAFLD patients can be diagnosed and staged effectively using noninvasive strategies even though liver biopsy can still be applied for individuals with dubious diagnostic tests or if noninvasive staging is unspecified [69]. However, there is no widely available simple blood test or imaging modality that can differentiate simple steatosis from NASH.

In summary, early diagnosis of NAFLD is essential to halting the progression of the disease. Biopsy is intrusive and therefore cannot be routinely applied. Ultrasound (sonography) and magnetic resonance imaging tools have become alternative noninvasive detection tools that can be routinely employed in clinical practice. The NAFLD activity score is important as part of the diagnosis procedure. But the fibrosis score is just as important. **Table 2** shows the fibrosis score currently used to stage the degree of fibrosis in the liver. There are a few noninvasive fibrosis imaging tests on the market such as Fibroscan that offers a liver stiffness measurement (LSM) using pulsed-echo ultrasound as a surrogate marker of fibrosis [70] and acoustic radiation force impulse (ARFI), which uses conventional B-mode ultrasonography to produce an ultrasonic pulse and measure the response of the liver tissue as shear wave velocity [71]. The Centers for Disease Control (CDC) and Prevention projects that diabetes mellitus is likely to impact the fibrosis progression rates, given the close link between diabetes and fibrosis in those with NAFLD [72, 73].

Some commercial biomarker tests include enhanced liver fibrosis (ELF), a panel of markers of matrix turnover as tissue inhibitor of matrix metalloproteinase 1 (TIMP1), hyaluronic acid and PIIINP [74] and FibroTest (FT), a panel of markers of fibrosis widely used in France.

Grade	Steatosis (%)	S score	Lobular (L) inflammation	L score	Hepatocyte ballooning (B)	B score
0	<5	0	No foci	0	None	0
1	5–33	1	<2 foci per 200 × field	1	Few cells	1
2	34–66	2	2–4 foci per 200 × filed	2	Many cells	2
3	>66	3	>4 foci per 200 × filed	3	N/A	N/A

NASH activity grade = total score: S + L + B range (0–8). Score of ≥5 is equivalent to NASH; score of 3 or 4 is borderline NASH; score of ≤2 denotes non-NASH NAFLD. The NAFLD activity score is based on three pathologic features: Steatosis, hepatocyte ballooning degeneration, and lobular inflammation. Higher scoring denotes severity of NASH: >5 = NASH; <5 = No NASH; 3–4 = borderline; none (0); few (1); many (2).

Table 1.
NAFLD activity score (NAS).

Liver injury	Fibrosis score (0–4)*	Fibrosis stage
None	0	0
Mild (delicate fibrosis)/zone 3 presinusoidal fibrosis	<5% (0), 5–33% = (1)	1a
Moderate (dense fibrosis)/zone 3 presinusoidal fibrosis	>33–66% (2), >66% = (3)	1b
Periportal/portal fibrosis	0 (0), <2 (1), 2–4 (2), > (3)	1c
Portal and periportal fibrosis/presinusoidal fibrosis	None (0)	2
Bridging fibrosis	Few (1)	3
Cirrhosis	Many (2)	4

**Fibrosis score of F1-F4 is generally considered NASH [4].*

Table 2.
NAFLD fibrosis score (NFS) and stage.

1.4 The metabolic syndrome (MetS)

Recognizing patients with the metabolic syndrome (MetS) is key to identifying patients at risk of NAFLD. MetS is a group of risk factors that raises risk of heart disease, diabetes, stroke, etc. [75] and is diagnosed when any three of the following five clinical risk factors are present [76]: impaired fasting serum glucose, low levels of serum HDL cholesterol, elevated serum triglycerides (i.e., hypertriglyceridemia), central obesity or larger than cut-off waist circumference (varies according to gender and ethnicity), and high blood pressure (HTN) (see **Table 3**).

Insulin resistance is also a major risk factor for the development of steatosis. Once considered benign, NAFL (or simple steatosis), which is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes, is now believed to be a serious risk factor for progression to liver disease, cardiovascular disease, and mortality [37, 77]. This is because an excess of abdominal fat is most tightly associated with the metabolic risk factors [78, 79]. The duration of obesity and the presence of MetS in an individual patient are closely tied to the risk of developing NASH-related cirrhosis and HCC [80]. Some of the characteristics of MetS are present in most NAFLD individuals, with 65–71% being obese, 57–68% having deranged lipid profiles, 36–70% suffering from HTN, and 12–37% having impaired fasting glucose tolerance [81]. Approximately a third of patients with NAFLD have the full metabolic syndrome and >90% have at least one feature [47]. There is a consensus that considers NAFLD as a hepatic manifestation of the MetS [82, 83]. On the other hand, clinical signs of the disease are manifested in 70–75% of T2DM patients and up to 95% of obese patients [84]. Thus, the development of the MetS, which is an important

Features	Terms of condition
Blood glucose (sugar)	Fasting, ≥100 mg/dL
Blood HDL ("good") cholesterol	♂ < 40 mg/dL; ♀ < 50 mg/dL
Blood triglycerides (TGs)	Fasting, ≥150 mg/dL
Waist circumference	♂ > 40″; ♀ > 35″
Blood pressure (HTN)	≥130/85 mm Hg

**The MetS is present with any three of the features shown in the table.*
♂ = male; ♀ = female; HDL = high-density lipoprotein; " = inches.

Table 3.
*Features of the metabolic syndrome.**

predictor of NASH in NAFLD patients, poses a sweeping and unfavorable prognosis [85]. IR is a key mediator that links NAFLD and MetS, which is a constellation of anthropometric and metabolic abnormalities (see **Table 3** above).

According to the latest data from NHANES (National Health and Nutrition Examination Survey) study conducted between 2011 and 2012, the prevalence of MetS has increased to 35% in American adults [86]. MetS is a risk factor for diabetes and cardiovascular diseases. It induces an abnormal production of hormones such as leptin, adiponectin, and cytokines such as TNF (tumor necrosis factor)-alpha that regulate inflammatory responses and cause disequilibrium between the pro-inflammatory and anti-inflammatory state of the organ [86]. These are mutually antagonistic: the pro-inflammatory factors such as TNF-alpha promote pro-apoptotic processes, recruit white blood cells, and promote insulin resistance. On the other hand, adiponectin acting as an anti-inflammatory factor inhibits fatty acid uptake, stimulates fatty acid oxidation and lipid export, and enhances insulin sensitivity. Both an increase in pro-inflammatory factors and a decrease in anti-inflammatory factors cause a cytokine imbalance that would lead to steatosis (NAFL) followed by necroinflammation (NASH) and IR. There is a supporting evidence that a high TNF to adiponectin ratio promotes fatty liver and steatohepatitis in animal [87] and human [88] studies. The importance of MetS including IR is that it predicts the occurrence of diabetes and cardiovascular diseases, which can further promote the development and progression of arteriosclerosis and HTN leading to significant morbidity and mortality [89]. Also, NAFLD and obesity are risk factors for the progression to fibrosis among HCV-infected patients [90–93]. Furthermore, elevated levels of ferritin are common in NAFLD patients and typically reflect active IR or underlying inflammatory activity [68, 81, 94]. Therefore, because of many different correlates and etiological factors and an assortment of assessment tools associated with MetS, there are some unresolved uncertainties in the current estimates of the global and the United States prevalence of NAFLD.

1.5 The genetics of NAFLD

Genetic disorders of lipid metabolism can cause hepatic fat deposition. However, they are far less common than excess body weight and features of MetS as risk factors for NAFLD and NASH. Several genes have been associated with NAFLD. These include *NCAN*, which may have a protective effect for Hispanics but increases risk of steatosis for non-Hispanic blacks; *LYPLAL1*, *GCKR*, as well as *PPP1R3B*, which may confer increased risk for hepatic steatosis but the data of distinctive serum lipid profiles in all these genes are sparse [61, 95–97]. *GCKR* is reported to be closely associated with NAFLD in Chinese [98]. Another gene, Patatin-like phospholipase domain-containing 3 (*PNPLA3* or adiponutrin), has emerged as the genetic factor predisposing Hispanics more at risk for fatty liver disease [99]. This adiponutrin gene is a single variant considered responsible for increased hepatic TG levels, fibrosis, and inflammation, observed among ethnic groups [100, 101]. Homozygote patients have a twofold rise in hepatic fat content than heterozygotes, and Hispanic populations exhibit the highest frequency of this polymorphism (49%) compared to 23% in European-Americans (EAs) and 17% in African-Americans (AAs) [101]. It also shows more allelic frequency with Hispanics than other ethnic groups. Romeo and colleagues [102, 103] along with Singal and colleagues published papers in 2008 in which they reported that *PNPLA3* is strongly associated with hepatic steatosis and elevated ALT and also recently showed that *PNPLA3* is associated with NASH, fibrosis progression, and hepatocellular cancer as well [102].

A genetic marker, *TM6SF2*, discovered in an exome-wide association study of liver fat content, has also shed some light on its association with hepatic steatosis. It

is involved in the loss of function mutation in very low-density lipoprotein (VLDL) secretion, and its association with NASH and advanced fibrosis has been recently validated even though its precise function has not been delineated [102]. However, its mutation is associated with elevated ALT, hepatic steatosis, and lower level of alkaline phosphatase, LDL, and TGs. This gene is most prevalent in European ancestry and less in Hispanics and AAs [104].

There is a reported 52% heritability rate of NAFLD, but evidence pertaining to specific genetic mutations is scant according to multivariable models used after adjusting for sex, age, and ethnicity [13]. Although the mechanism is not well understood, genetic mutations in hemochromatosis (HFE) gene, which is responsible for iron uptake and transferrin plasma concentration, may also be associated with NAFLD development [105, 106]. Several other factors have been indicated in the development and outcomes of NAFLD including epigenetic alterations [107, 108], maternal perinatal nutrition [109–111], and gut microbiota [107, 112–114]. A recent study also reports a novel pathway in which hepatic vitamin D receptor (VDR) expression is increased in patients with simple steatosis (nonalcoholic fatty liver without inflammation), and the activated VDR upregulates angiopoietin-like protein 8 (ANGPTL8) expression, thus contributing to triglyceride accumulation in human hepatocytes [115]. At any rate, studies have reported that fibrosis-initiated fatty liver disease progresses over many years, thus providing a potential window for intervention by examining disease-progression/modifying factors in NAFLD [116–118]. It is important to note that increased BMI and insulin resistance have been associated with a more rapid progression to fibrosis [35, 119].

1.6 The epidemiology and prevalence of NAFLD

In most epidemiological studies, the prevalence of NAFLD in the general population is determined by imaging or other indirect methods. Accordingly, the epidemiology and demographic characteristics of NAFLD vary worldwide [12, 16]. In epidemiological studies, the pathophysiological aspects, the natural history, and the determinants of NAFLD are important parameters for the diagnosis and evaluation of therapeutic interventions. This section will provide global perspectives on the prevalence of NAFLD (and later HCC) with emphasis on the United States and the possible reasons for the rapid rise.

There are wide-ranging estimates of NAFLD prevalence in the general population of the United States. An estimated 17–51% of adults have NAFLD [23, 120, 121]. Analysis of liver ultrasound data collected between 1988 and 1994 from the NHANES III reported that 19% of adults have NAFLD [122], whereas a meta-analysis of studies from 2006 to 2014 estimated a NAFLD prevalence of 24% (20–29%) in the general population [65]. The prevalence of NASH is difficult to estimate as biopsy is the necessary tool for screening, but it is cost-prohibitive and impractical for a population study.

Globally, NAFLD is a growing cause of chronic liver disease and NASH is replacing HCV infection as the primary reason for LT [13, 123, 124]. The broad category of NAFLD can manifest as NAFL or NASH. Fibrosis precedes cirrhosis and is therefore used as a prognosticator of the clinical risk of progression to cirrhosis and long-term liver-related adverse outcomes and mortality [34]. Recent evidence has shown that NAFLD and NASH can progress to HCC even in the absence of cirrhosis [125–127]. In most epidemiological studies including the NHANES data set, the assumptions about NASH in the NAFLD population are based on a post-hoc application of liver enzymes (i.e., AST and ALT) and clinical measurements. In the same vein, the fibrosis stages in population-based studies reflect best estimates derived

from clinical aids (e.g., fibrosis-4, ALT to platelet ratio index, and NAFLD fibrosis scores) [128, 129]. The current prevalence rates for NAFLD, NASH, and HCC based on definitive clinical manifestations are shown in **Table 4**.

Certain risk factors such as advanced age, obesity, ethnicity, and T2DM increase the incidence and prevalence of NAFLD and NASH and have been consistently identified as salient risk factors for fibrotic progression to cirrhosis [130] (see **Table 5**).

The current global estimate is that 24–30% of the world's population is affected by NAFLD [65] and that includes between 80 and 100 million Americans (http://www.mayoclinic.com), making it the primary etiology for liver disease in the United States; see **Figure 2**.

The increasing incidence of obesity, diabetes, and metabolic syndrome in the United States and Europe may soon catapult NAFLD/NASH to become the most common cause of HCC in developed countries. In the United States, among the more than 26 million people with diabetes, the prevalence of biopsy-proven NAFLD and NASH is as high as 74 and 11%, respectively [138, 139].

1.7 The rise in burden of NAFLD/NASH

The global rise of NAFLD has exasperated the looming healthcare burden of disease. It may be difficult to accurately forecast the current and future burden of a disease that is rapidly progressing. However, there are modeling techniques and approaches that incorporate real-world surveillance data for NAFLD and NASH incidences, which are growing causes of cirrhosis and HCC. As with many models, the utility of the model is linked to the validity of the inputs into the model. One of these modeling approaches is based on the premise that public awareness and

Status	Definition	Prevalence	Prognosis
NAFLD	Spectrum of fatty liver disease with <140 g for men and < 70 g for women per week of alcohol consumption	Estimated at 24–30% of global population [13, 14, 16] and at least 31% of US population (7)	—
NAFL	>5% simple hepatic steatosis by weight of liver without evidence of hepatocellular injury (i.e., hepatocyte ballooning)	>80% of NAFLD patients	Low probability of progression to cirrhosis
NASH	>5% hepatic steatosis by weight of liver with inflammation and hepatocellular injury with or without fibrosis Confirmed histologically	Estimated at up to 21–59% of patients with NAFLD Estimated at 1.5–6.45% of US general US population [40, 96, 122]	11% progress to cirrhosis over 15 years
NASH cirrhosis	Presence of cirrhosis with current or past histologic evidence of steatosis	10–30% of patients with NASH	About 31% have liver decompensation over 8 years; about 7% develop HCC over 6.5 years
NASH HCC	Hepatocellular carcinoma induced by NASH	Estimated at annual rate of 2.6–12.8%*	Progresses to end-stage liver disease

**The prevalence of NASH-HCC is not firmly established. Data in the table are the annual incidence rate of developing HCC in patients with NASH-related cirrhosis.*

Table 4.
Prevalence of NAFLD and its more progressive forms, NASH and HCC.

Risk factor	Description	References
Age	Risk increases with age	[4, 40]
Gender	More common in men Higher risk of advanced fibrosis in women	[4, 96, 131, 132]
Genetics	Patatin-like phospholipase domain-containing 3 gene	[133]
MetS**	70–90% of patients have NAFLD MetS is an independent predictor of fibrosis	[85, 95]
Ethnicity	Elevated risk in Hispanics Lower risk in blacks	[61]
Diet	Elevated levels of cholesterol and saturated fats High fructose intake, low carbohydrates	[89, 134–136]
OSA***	Increased risk of hepatic fibrosis	[137]

**NAFLD/NASH = nonalcoholic fatty liver disease/nonalcoholic steatohepatitis.*
***MetS = metabolic syndrome.*
****OSA = obstructive sleep apnea.*

Table 5.
NAFLD/NASH risk factors.*

government health policies will be able to eventually level off national obesity incidences and prevalence, which in return will level off NAFLD [4]. The interpretation of the output of this and other models attempting to analyze the burden of NAFLD is constrained by the lack of accurate diagnosis of steatohepatitis with simple epidemiologic tools. Nevertheless, the proportion of individuals with NASH in the NAFLD population will probably continue to rise through the next 15 years based on the rising prevalence of diabetes mellitus [4].

Analyzing the cost and burden of disease with respect to NASH has several potential implications. First, it helps introduce strategies and treatment regimen that will stem its exponential rise in incidence and mortality rates; it will reduce the growing contribution of NASH to LT, which is expensive; and due to an oversupply of decompensated cirrhosis, matching organ availability is rare, and insurance companies have exclusive policy of qualifying subjects with NASH-induced cirrhosis based on whether they have associated co-morbidities.

The epidemiology and demographic characteristics of NAFLD vary worldwide. The rise in NAFLD and NASH will balloon the number of patients with decompensated cirrhosis and pose a major emotional and financial burden on subjects and

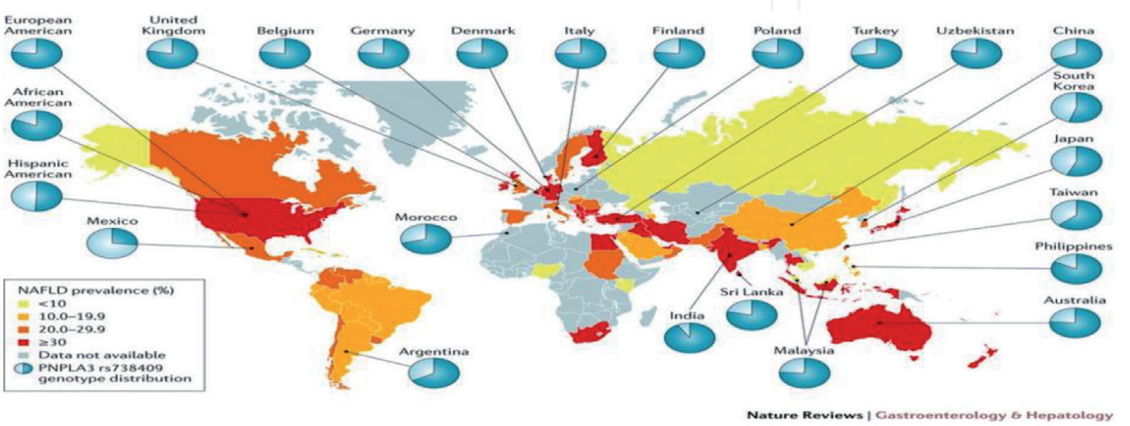

Figure 2.
Global picture of estimated prevalence of NAFLD and distribution of PNPLA3 genotypes adopted from Zobair Younossi [16]. PNPLA3 is presented as minor allele frequency in some areas (light blue section of the pie chart).

their caregivers, thus adding to the overall cost of health care. Furthermore, the main etiologic factor adding to the burden of HCC is NAFLD [4]. In select NASH-related HCC patients, liver resection and transplantation provide potentially curative therapeutic options; however, these procedures place a significant burden to health-care resources and utilization [140]. Currently, NASH-related HCC has replaced HCV-related HCC as the fastest growing indication for LT in HCC candidates.

2. Hepatocellular carcinoma (HCC)

Liver cancer, which has limited therapeutic choices, has the second highest mortality rate in the world [141]. HCC, which can lead to complications such as portal vein thrombosis (PVT), accounts for the majority of primary liver malignancies and is one of the leading causes of death in patients with advanced fibrosis or cirrhosis [141–144]. HCC can be caused by chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), alcohol abuse, as well as obesity and diabetes-induced MetS. NAFLD often occurs in the setting of metabolic disorders such as obesity and T2DM. These same metabolic conditions are also risk factors for NAFLD-associated HCC, which can materialize in individuals even in the absence of advanced fibrosis or cirrhosis. NASH-HCC appears to be phenotypically different from HCC arising from other chronic liver diseases (**Table 6**). By all accounts, the formation and progression of HCC are multistep processes. Therefore, the specific and detailed molecular events that underlie HCC development remain only partially understood [143].

2.1 The epidemiology and prevalence of HCC

Primary liver cancer in 2012 was identified as the second most common cause of cancer-related death in the world. In the United States, HCC is the most common histological subtype of liver cancer that accounts for 70–85% of primary liver malignancies [145, 146]. It is also the most rapidly rising cause of cancer and

Hepatitis B infection (HBV)
Hepatitis C infection (HCV)
Hepatitis D infection (HDV)
Alcohol (ethanol)
Nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH)
Obesity/diabetes
Hereditary hemochromatosis
Aflatoxin B
Tyrosinemia
Glycogen storage disease type 1a
Oral contraceptives
Smoking
Cirrhosis*

**Diagnosis of cirrhosis is based on the presence of the ICD-9 codes for cirrhosis or complications of cirrhosis (gastroesophageal varices, encephalopathy, and nonmalignant ascites) recorded at least twice in any inpatient or outpatient encounter.*

Table 6.
Common risk factors for hepatocellular carcinoma.

cancer-related deaths with an incidence that has more than tripled over the last two decades. This high mortality reflects a poor prognosis and a poorer therapeutic intervention [147]. Compared to HCC caused by alcoholic liver disease and viral hepatitis, there is a lack of strong epidemiological data associated with the incidence and prevalence of HCC precipitating from NAFLD [140, 148]. While the prevalence of NAFLD is thought to be highest among Hispanics and Caucasians, the ethnic distribution among NAFLD-/NASH-related HCC patients has yet to be defined. Male patients are overrepresented in NASH-related HCC; however, gender has not been proven to be a statistical risk factor in NASH progression to HCC [7]. The rising incidence of NAFLD/NASH in the setting of obesity has led to a drastic growth in NASH-related HCC incidence [149]. Although NAFLD can present with HCC in the absence of NASH or cirrhosis, the cumulative annual incidence rate for developing HCC in patients with NASH-related cirrhosis is approximately 2.4–12.8% [125]. This suggests or utmost underlies that cirrhosis may be the main cause of HCC despite new emerging data suggesting that NAFLD may be an independent risk factor for HCC, even in the absence of cirrhosis [126, 150, 151].

There was also a twofold increase in the incidence of HCC in the United States over the past two decades, and it is projected to double over the next two decades. Compared to HCC in alcoholic liver disease and viral hepatitis, there is a lack of strong epidemiological data regarding the incidence and prevalence of HCC in NAFLD [148]. It is projected that in just 12 more years, HCC at its current pace of growth in the United States will outstrip breast and colorectal cancers as the third leading cause of cancer-related death. This is because the prevalence of HCC is expected to increase by 149% from 10,000 to 24,900 during 2015–2030, while the incidence of HCC cases is expected to increase from 5160 to 12,240 in 2030, an increase of 137% [4]. This alarming incidence is attributed to several different genetic and epigenetic alterations that are under investigation [4].

Modeling the epidemic of HCC suggests that in 2015, 3280 incident HCC cases were estimated to have progressed from compensated cirrhosis (64% of total), with the remaining 1880 incident cases occurring among ≤F3 (fibrosis score-3) cases [4]. By 2030, 8790 incident HCC cases are predicted to occur among compensated cirrhotic cases or 72% of the annual incidence, reflecting aging and disease progression [4].

The true prevalence of NASH and NASH-related HCC is probably underestimated. This is because in 6.9–29% of HCC cases, the underlying etiology is unknown, further questioning the designation that the liver disease is secondary to cryptogenic cirrhosis [148]. Traits of NASH are more frequently observed in HCC patients with cryptogenic cirrhosis than in age- and sex-matched HCC patients of well-defined viral or alcoholic etiology [152]. In the past several years, myriad studies have tried to determine the variability of relationships between NAFLD/NASH, cryptogenic cirrhosis, and HCC. In a recent meta-analysis, White et al. [125] estimated that 60% of HCC cases ascribed to NAFLD/NASH had cirrhosis either prior to diagnosis or at the time of diagnosis. This same analysis showed that NASH-associated cirrhosis consistently manifested an increased HCC risk. Furthermore, the study also revealed that when compared to those with chronic HCV, the risk of developing HCC is lower in patients with cirrhosis due to NAFLD/NASH (HCV, 19.7% vs. NAFLD/NASH, 26.9%) [125]. Although the prevalence of NAFLD-/NASH-related HCC is not well delineated, the growing incidence of obesity and diabetes suggests the impact of NAFLD-/NASH-related HCC will continue to grow.

2.2 The genetics of HCC

Genomic analyses promise to improve tumor characterization for the optimization of precision or personalized medicine for patients with HCC. Recent

developments and molecular techniques have significantly improved our understanding of the pathogenesis of HCC and its complex genetic landscape [153–156]. The integration of several profiling data from various sources may provide additional insight into the molecular mechanisms of HCC [153]. The first large-scale multiplatform analysis of HCC conducted as part of The Cancer Genome Atlas (TCGA) network included valuation of somatic mutations by whole exome sequencing and DNA copy number analyses in 363 patients whose tissue and tumor specimens were obtained [157]. This high-throughput analysis also included further investigation of DNA methylation, mRNA expression, microRNA (miRNA) expression, and proteomic expression in 196 patients. To decipher the molecular landscape of HCC and extract biological insights for therapeutic targets and prognostic implications, analyses were made by integrating multiple data platforms with the available clinical data for HCC [157]. Mutational and DNA sequencing analyses identified an array of genes altered either by downregulation or by mutation. Among the significantly mutated genes were EEF1A1, SMARCA4, LZTR1, and SF3B1 [157]. Those genes downregulated by hypermethylation including ALB, APOB, and CPS1 may cause metabolic reprogramming in HCC. The analysis of integrated molecular platform also yielded the identification of a subtype linked to poorer prognosis in three HCC cohorts. This large-scale multiplatform, high-throughput analysis enabled the design of a p53 target gene expression signature correlating with poor survival. This TCGA network analysis produced potential therapeutic targets including WNT signaling, IDH1, MET, VEGFA, MCL1, MDM4, TERT, and immune checkpoint proteins PD-1, PD-L1, and CTLA-4 [157]. This is significant because effective inhibitors already exit for these targets, which alter hepatocyte energy balance [157].

In exome sequencing analysis of over 200 liver tumors, investigators identified mutational signatures that are associated with specific risk factors such as alcohol and tobacco consumption and exposure to aflatoxin B_1 [158]. As a result, they found that 161 putative driver genes were associated with 11 recurrently altered pathways involving *CTNNB1* (alcohol), *TP53* (hepatitis B virus, HBV), and *AXIN1* [158]. Further analysis of tumor stage progression identified *TERT* as an early event, whereas *FGF3*, *FGF4*, *FGF19*, or *CCND1* amplification and *TP53* and *CDKN2A* alterations were prominent in aggressive tumors. The involvement of these many altered genes and pathways in the development and/or progression of HCC leads to the extensive landscape and multifaceted nature of this lethal cancer. **Figure 3** shows the salient signaling pathways associated with HCC.

In another recent study, gene expression and DNA methylation profiles were screened to identify potential genetic biomarkers of HCC. The findings from this study suggest potential HCC biomarker roles for certain genes such as *DTL, DUSP1, NFKBIA*, and *SOCS2* [160]. Similar to TCGA Research Network analyses mentioned above [157], these investigators also suggest that the tumor protein 'p53 signaling' and 'metabolic' pathways may serve important roles in the pathogenesis of HCC [160]. Other polymorphic variants serving as potential risk factors for HCC in high-risk patients infected with HBV/HCV have also been reported [161]. As for prognostic biomarkers, recent RNA sequencing data from the Cancer Genome Atlas (TCGA) reveal that among the 12 tissue types studied, the liver had the largest number of tissue-enriched genes, which are associated with the prognosis of patients with HCC and represent distinct physiological patterns [162]. A further study of the characteristics of liver-enriched genes showed that hypermethylation might be partially responsible for the downregulation of these genes, most of which were metabolism-related genes associated with pathological stage and dedifferentiation in patients with HCC. The authors suggest that hypermethylation might be a mechanism

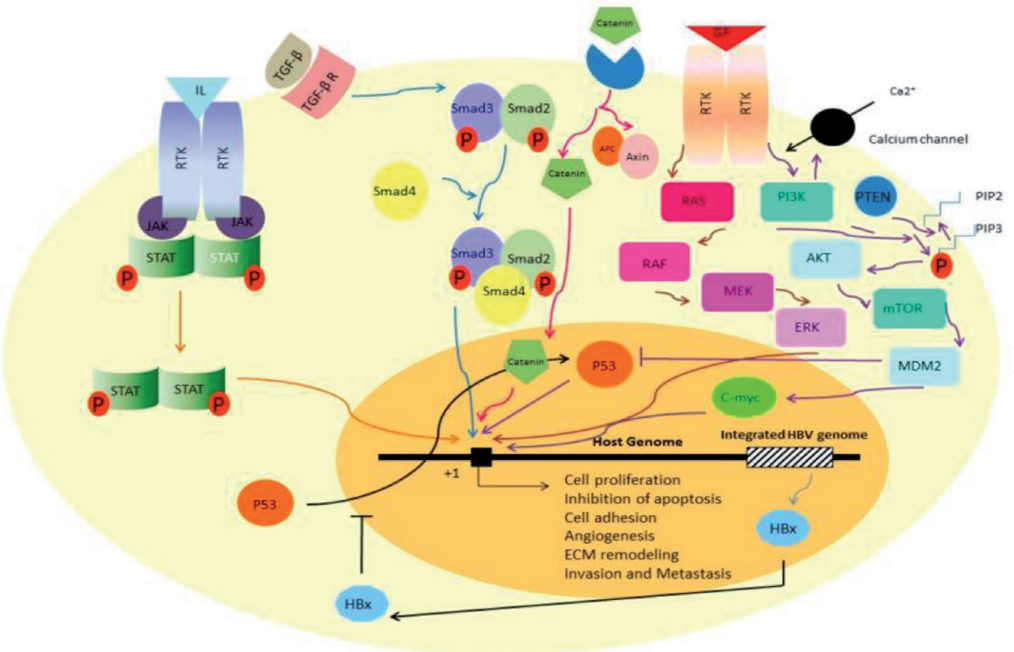

Figure 3.
The most reported signaling pathways in HCC. Adapted from Birgani et al. [159].

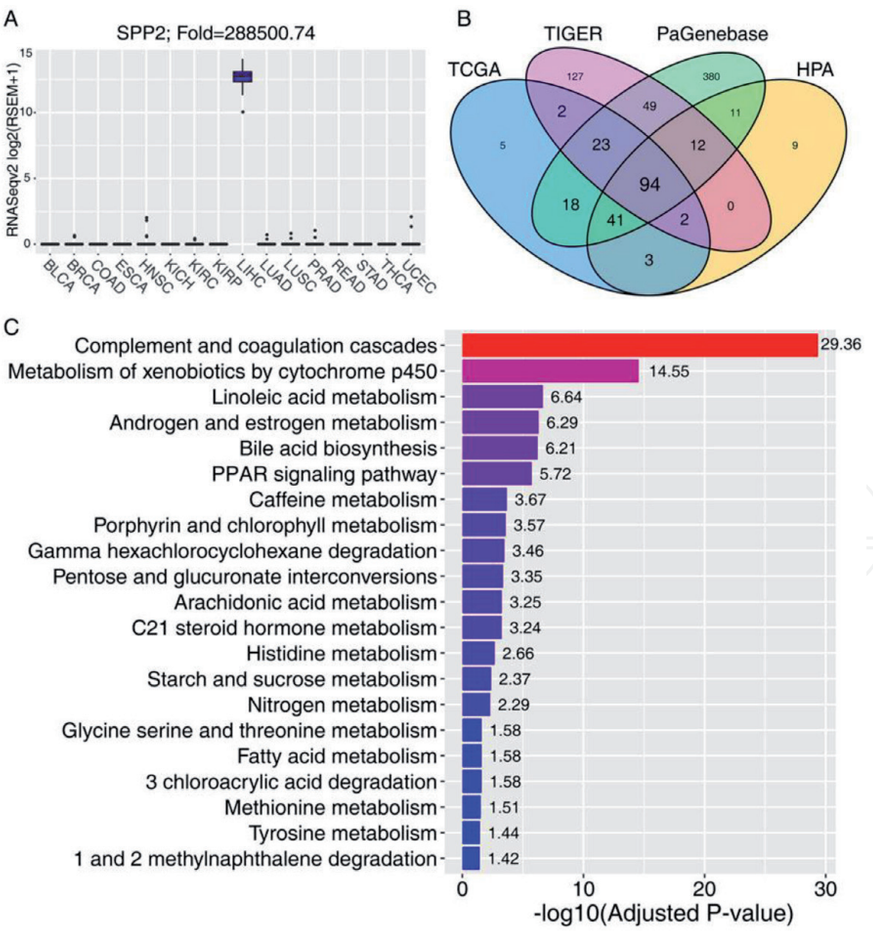

Figure 4.
Validation of liver-enriched genes and KEGG analysis. (A) an example of liver-enriched genes. SPP2 was exclusively expressed in the corresponding nontumor tissues of HCC. (B) Four-set Venn diagram showing the overlap of the liver-enriched genes derived from the TCGA and three other databases, including HPA, PaGenBase, and TiGER. (C) Significantly enriched KEGG pathways of 188 liver-enriched genes. –log10 (adjusted p-value) was annotated on each bar of the KEGG pathway. Adapted from Binghua et al. [162].

underlying the downregulation of these liver-enriched genes. When they overlapped the tissue-enriched and prognostic genes across cancer types, they found that, in HCC, 55% (84/188) of the liver-enriched genes were prognostic (see **Figure 4**).

Circulating regulatory nucleic acids like miRNA profiles can also reflect the pathogenic changes occurring in organs including the liver. Changes in miR-21, miR-122, and miR-223 were correlated with the histological status of the human liver and were specific for liver injury [163]. These miRNA levels were significantly higher in the serum of chronic hepatitis (i.e., HBV and HCV) and HCC patients compared to healthy controls [44]. Yet, the biological heterogeneity of HCC makes it difficult to clarify the key mechanisms of cancer initiation and progression, and thereby develop and implement effective therapies [164].

3. The projection of NAFLD and HCC

A recent Markov model was used to predict incidence of NAFLD and to forecast NAFLD disease progression in the United States. The model was based on historical and projected changes in adult prevalence of obesity and T2DM as well as national surveillance data for incidence of NAFLD-related HCC [4]. The report forecasts that prevalent NAFLD cases will increase to 21% (100.9 million) by 2030, while prevalent NASH cases will increase 63% from 16.5 million to 27.00 million cases [4]. Overall NAFLD prevalence among the adult population (aged ≥15 years) is projected at 33.5% in 2030, and the median age of the NAFLD population will increase from 50 (estimated at 2015 level) to 55 years between 2015 and 2030 [4]. In 2015, approximately 20% of NAFLD cases were classified as NASH and are expected to increase to 27% by 2030, a reflection of both disease progression and an aging population. The estimated prevalence of NASH in adults living in the United States is 3–5% [6, 23, 121, 165] and is projected to increase by 63% from 16.5 million in 2015 to 27.00 million cases in 2030 [4]. This prevalence of NASH was calculated based on published estimates and modeling of fibrosis progression. It was assumed that up to 5% of NAFLD cases without NASH could be NASH regressors, with most NASH regressors still in F0 stage [4]. Similarly, the incidence of decompensated cirrhosis will surge by 168% to 105,430 cases in 2030, while incidence of HCC will increase by 137% to 12,240 cases. Liver deaths are estimated to increase 178% to 78,300 deaths in 2030. During 2015–2030, there are projected to be nearly 800,000 excess liver deaths. The aging population, the continuing high rates of adult obesity, and T2DM will propel NAFLD-related liver disease and mortality in the United States. Immediate strategies are required to curtail new NAFLD cases and mitigate disease burden.

Currently, NAFLD is estimated to affect more than 80 million and up to 90 million Americans, making it the most common etiology for liver disease in the United States [16, 65]. In the United Kingdom, NAFLD has now become the most common cause of abnormal liver function tests (LFTs) [166]. Although NAFLD has emerged as a serious disease in affluent Western economies, its global prevalence encompasses the Middle East (32%), South America (31%), Asia (27%), the United States (24%), Europe (23%), and Africa (14%) [167]. Because of the increasing incidence of obesity and diabetes around the world, NAFLD has become a global public health concern. The prevalence of NAFLD varies according to age, sex, and the methodology used to measure the condition in each geographical location [61]. Currently, NAFLD is the most prevalent liver disease observed in patients with obesity, diabetes, and metabolic syndrome (MetS), all of which can confer insulin resistance (IR) and are known risk factors for the development of HCC, a growing indicator of LT [45, 69]. While HCV infection has been the most common indication for liver transplants to date, NASH is surpassing it as obesity reaches historic

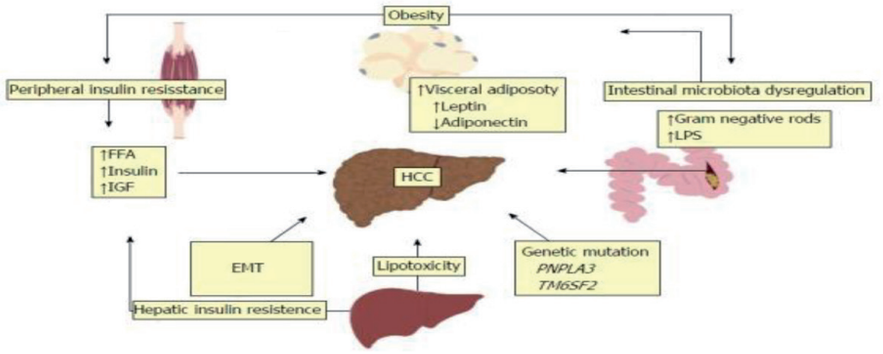

Figure 5.
Risk factors and proposed mechanisms for NAFLD- and NASH-related HCC, which is multifactorial. Proposed pathogenic mechanisms include obesity, peripheral and hepatic IR from T2DM, increased hepatic lipid storage and lipotoxicity, genetic mutations, and intestinal microbiota dysregulation. HCC, hepatocellular carcinoma; EMT, epithelial to mesenchymal transition; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; FFA, free fatty acid; IGF, insulin-like growth factor; LPS, lipopolysaccharide; PNPLA3*, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6 superfamily member 2. Adapted from Cholankeril [140].*

highs and new direct-acting antiviral (DAA) drugs are essentially curing hepatitis C [168]. Furthermore, with the continued decline in the prevalence of HCV infection, the proportion of NASH-HCC is anticipated to increase exponentially due to the growing epidemic of obesity and diabetes [140]. Currently, NASH-related HCC is the fastest growing indication for LT in HCC candidates [140]. NAFLD and NASH are a growing cause of cirrhosis and HCC.

Globally, Asia is leading the rise in NAFLD followed by the United States. Although our understanding of NAFLD is steadily evolving, it is not an isolated disease. It is commonly associated with the leading metabolic comorbidities such as obesity, MetS, T2DM, and dyslipidemia. The potential progression of NAFLD subtypes is from fibrosis to advanced fibrosis, ESLD, and HCC (**Figure 1**). As the incidence of obesity and concurrently diabetes and MetS continues to surge in Europe and the United States, NAFLD/NASH may become the most common cause of HCC in developed countries in the foreseeable future [169–171].

A 2002–2012 retrospective cohort study among adult patients revealed a four-fold increase in patients undergoing LT for NASH-related HCC in contrast to only twofold increase in number of patients undergoing transplantation for HCV-related HCC. In the United States, about 6000–7000 liver transplants are performed annually, and the rapid increase in the percentage (44.9%) of obese individuals during a 14-year period (2000–2014) is expected to escalate to 55% the number of NASH patients awaiting LT by 2030 [172]. The increased morbidity and mortality, healthcare costs, and declining health-related quality of life associated with NAFLD require more in-depth analysis. **Figure 5** depicts the proposed mechanisms that ties NAFLD/NASH and HCC.

4. Ethnic and gender differences in NAFLD and HCC

Although still not fully resolved, the prevalence of NAFLD in the United States can vary by ethnicity. Even in this context, there are several factors that could explain the reported ethnic disparities. These include access to health care, genetic factors, environmental factors, affliction with chronic diseases, and the presence of chronic diseases such as the MetS [61, 65, 173]. In this context, the prevalence of NAFLD is reported to be highest in Hispanic-Americans, followed by Americans of European descent and then African-Americans [40, 61, 65, 122, 173]. Several

studies have shown a relative sparsity of NAFLD cases among individuals of African descent living in or coming from Africa or the Caribbean region. Although the prevalence of metabolic disease and obesity is high in Afro-Caribbean ethnic groups compared to Caucasian and Hispanic groups, the frequency of NAFLD/NASH is reported to be low [61, 174]. This discrepancy might be due to an actual low number rate or biases that include low-recognition and low-referral rates in these ethnic minorities [175], as Afro-Caribbean patients are categorically less likely to be referred to other tertiary hospitals [176].

There are also ethnic differences in the incidence of HCC in the United States (see Sherif et al. for a comprehensive review) [61]. Compared to European-Americans (EAs), the incidence of HCC is higher in African-Americans (AAs) and is associated with more advanced tumor stage at diagnosis and lower survival rates overall. Assessment of changes in the levels of metabolites of samples stratified by race was made using gas chromatography-mass spectrometry in selected ion monitoring mode to identify ethnically diverse biomarkers in HCC between EA and AAs [177]. Race-specific metabolites including alpha tocopherol for AA and EA combined, glycine for EA, and valine for AA exhibited better sensitivity and specificity than the standard serological marker for HCC, alpha-fetoprotein (AFP) that is widely used for the diagnosis of HCC [177–180]. It is hypothesized that there is a variation in HCC-associated epigenetic modifications between AAs and EAs. Thus, the identification of aberrant DNA methylation and differentially modulated miRNAs can be used to better understand the mechanisms of disparities in HCC between races. Also, identifying epigenetic markers for HCC in a specific population will enhance personalized medicine that targets specific therapeutic approaches [181, 182]. This also demands the gathering together of a highly interdisciplinary team of experts to investigate changes in both DNA methylation and miRNA expression patterns between tumor, cirrhotic, and normal liver tissues from AA and EA participants. Identifying molecular cancer gene drivers and mutations may 1 day become critical for precision oncology.

Most epidemiological studies document prevalence of individual diseases in selected tertiary hospital populations [183]. This widespread practice, particularly when imaging and liver enzyme tests are involved and when the patients may be asymptomatic in the early stage of diagnosis, leads to underestimation and underdiagnosis of NAFLD. This is especially true for minority populations in whom the natural development and progression of NAFLD and NASH are understudied and underreported as reflected by the paucity of data in the literature. Furthermore, the predictive value of the MetS may not reflect the true state of NAFLD in AAs since the criteria for the syndrome were developed for non-Hispanic whites [184] thereby influencing underdiagnosis or misdiagnosis of NAFLD and NASH in Hispanics and non-Hispanic blacks (NHB). There is also a strong relationship between insulin resistance and hypertriglyceridemia, one of the crucial components of MetS. However, NHB often have normal triglycerides (TG) level [185], which is used as a diagnostic criterion of the MetS leading to underdiagnosis of the MetS in NHB [186]. This suggests that lowering the threshold for TG level in AAs will lead to grasping the true cases of NAFLD. Moreover, the racial differences in NAFLD and NASH may be a function of the differences in TGs or the differences in the distribution of adiposity (e.g., subcutaneous vs. visceral) since AAs have relatively less VAT and lower TGs than Hispanics [119, 173, 175]. In addition, AAs may be more resistant to both the accretion of TG in the abdominal visceral compartment (adipose tissue and liver) and hypertriglyceridemia associated with IR [119].

Epidemiologic studies establish the foundational framework for the control and prevention of diseases. In the case of NAFLD and NASH, it should be done by first tracking the prevalence of the disease, characterizing its natural history, and

identifying both its social and health determinants along ethnic lines. This type of study is critical for the proper diagnosis and early intervention of NAFLD especially in minority populations [61].

Genome-wide association studies have revealed several genetic variants that are associated with NAFLD and NASH. Yet, these variants either represent only a limited amount of variation in hepatic steatosis among ethnic groups or may just be markers representing a larger body of genetic variations.

There is an urgent need to gain a better understanding of the underlying biological mechanisms responsible for why some people with NAFLD are more prone to developing HCC, and the causes for disparities in NAFLD-related HCC. There is also an urgent need for a less invasive method than biopsy and for a more sensitive biomarker than ALT for large-scale NAFLD screening. The lack of high-throughput studies employing proteomics or metabolomics for the discovery of novel and reliable diagnostic biomarkers for NAFLD also hampers our understanding of the pathophysiology of the disease among the disparate ethnicities [12, 177].

One recent area of exploration is the involvement of DNA methylation and miRNA regulation. Epigenetic alterations are potentially reversible, and this possibility will facilitate the development of biomarkers and therapeutics in the prevailing disparities between AA and EA patients in HCC initiation and development. The identification and functional validation of race-specific methylation hotspots and miRNAs can be used to understand the mechanisms of disparities in HCC. This can be done by first identifying DNA methylation sites and miRNAs with statistically significant changes between HCC cases and cirrhotic or normal controls in a race-specific manner. Then, network-based methods and hierarchical integrative models can be used to integrate epigenomic data with transcriptomic, proteomic, glycoproteomic, and metabolomic data acquired from the same cirrhotic and HCC participants to select methylation hotspots and miRNAs relevant for understanding the mechanisms of disparities in HCC [177]. The selected candidates can then be validated by independent methods using frozen and formalin-fixed, paraffin-embedded (FFPE) liver tissues collected from patients with HCC and liver cirrhosis. Finally, functional validation of race-specific epigenetic modifications discovered in this type of high-throughput study can be performed through *in vitro* experiments using established cell lines derived from racially diverse populations. These cell cultures may present unique opportunities for targeted functional validation of epigenetic modifications and the downstream consequences.

In addition to exploring the external environment and how it influences HCC disease status, it is also necessary to explore the intestinal environment of different ethnicities. Experimental data from the obesity epidemic have revealed that the composition and products of the gut microbiome, which is altered with obesity and/or a high fat diet, are carcinogenic to the liver [187, 188]. Studies suggest that there are ethnic differences in microbial composition in a cirrhotic population at elevated risk for HCC as a result of metabolites, which can differentiate cirrhotic with HCC from those without HCC. Therefore, a case-control study can be designed to examine the contributions of race/ethnicity, fecal microbiome, fecal metabolome, and host factors (e.g., specific dietary factors and markers of body and liver fat composition) to NAFLD-related HCC. All in all, a multiethnic study of NAFLD and HCC that encompasses all racial/ethnic groups is needed to lay the groundwork for the elucidation of factors that account for health disparities across these populations. The prevalence of NAFLD is reported to be highest among Hispanics and Caucasians as mentioned above. However, NASH was the leading cause of waitlist LT registration in 2016 among Asian, Hispanic, and non-Hispanic white females, whereas HCV is still the leading cause in AA females [189].

As for gender differences in NAFLD or NASH, there are uncertainties including the role of IR in the influence of gender on NAFLD. Ruhl et al. reported that NAFLD is about 2.7 times more prevalent in men than in women [190]. One reasonable explanation for this reported gender difference in NAFLD is due to the higher waist-to-hip circumference (WHR) ratio in men [96]. Pan et al. further state that WHR is associated with visceral adipose tissue (VAT), which is correlated with both peripheral and hepatic IR. Similarly, in the Dallas Heart Study, European-American (EA) men had an approximately twofold higher prevalence of hepatic steatosis than EA women. This gender disparity has been blamed on alcohol use, sex hormones or lifestyle behaviors, and no differences in body weight or insulin sensitivity [96].

The ethnic distribution among NAFLD-/NASH-related HCC patients has yet to be defined [191]. If the increase in the number of ethnic groups waitlisted for LT from 2004 to 2016 is a good indicator of the rise in NASH-HCC, then it could be inferred from a recent retrospective study that Asian females had an 854% change in NASH waitlist registration, while Asian males had a 552% change [189]. The increase in African-American waitlist population was much less compared to the other ethnic groups. In contrast, the Hispanic females had a 3010% change in the rate of waitlist registration for NASH with HCC, while non-Hispanic white females had a 1992% change [189].

NASH-related HCC patients are primarily male even though gender is not a proven statistical risk factor in the progression of NASH to HCC. However, NASH is currently the second leading cause for LT waitlist in females, whereas in men, alcoholic liver disease (ALD) continues to be the leading cause [189]. Although old data of 698 patients from biopsy-proven NASH show that NASH patients are more likely to be female than male possibly reflecting a higher disease burden rate in women [192], it is likely that both gender and racial ethnic differences in NAFLD and NASH are attributed to interaction among genetic, environmental, and lifestyle behaviors.

5. Medical therapy for NAFLD and HCC

The biological heterogeneities of NAFLD and HCC create predicaments in deciphering the key mechanisms of development and progression from NAFLD to ESLD. Although progress is being made in understanding the molecular underpinnings of chronic liver disease and its various offshoots, there are still formidable challenges in providing effective treatment regimens. Aside from a few prophylactic agents that have shown promise in the prevention and treatment of steatohepatitis and fibrosis, there is no treatment consensus due to scarcity of data [140]. Wholesome lifestyle and behavioral changes that include regular physical activity, low caloric intake, and weight loss are the main bulwarks against NAFLD, which may progress to HCC with or without cirrhosis. However, the extent to which these modifications are effective to prevent the development of HCC is unclear. There is currently no effective chemoprevention to decrease the incidence of HCC except using nucleoside analogs to reduce viral replication for those infected with HBV [193] and direct-acting antivirals (DAAs) for those infected with HCV [194], the latter demonstrating very high cure rates but also raising concerns about the recurrence or development of HCC after the achievement of a sustained virological response [195]. Obeticholic acid (OCA), a selective agonist of the Farnesoid X receptors, was touted to be a promising pharmacological drug for the management of NAFLD. However, its low efficacy and specificity have dampened enthusiasm for its practical use. Also, the drug pioglitazone has no long-term impact on NASH. This entails a pressing need to develop more effective and safe agents for NAFLD and HCC. Several other experimental studies suggest a direct role for vitamin D in

modulating liver fibrosis and inflammation by enhancing hepatic response to insulin via binding to vitamin D receptor on liver cells [196–198]. Vitamin E and carotenoids are also shown to decrease plasma levels of patients with NASH [199], whereas dietary antioxidants such as vitamin C and coenzyme Q12, trace minerals such as selenium, anticholesterol medications such as statins, antidiabetic drugs such as metformin, and methyl radical donors such as S-adenosylmethionine have all been touted as potential prophylactic agents [169, 200–202].

6. Key findings, future trends, and unmet needs

Hepatic steatosis is associated with many other morbidities. Therefore, dissecting the myriad causative agents including genetic, hormonal, or environmental factors underlying the pathogenicity of simple hepatic steatosis must be a priority to avoid the maze of complications that may arise during the development of NAFLD and its progression to HCC. The key findings are:

- Global prevalence of NAFLD is at 24% but is rising to greater than 30%; highest rates to lowest rates are found in South America, Middle East, Asia, United States, and Europe.
- The large volume of patients sets NAFLD apart from other liver diseases; thus, clinical care must focus on discerning highest risk of progressive liver disease.
- Overweight in childhood and adolescence is associated with the risk of NAFLD later in life and increases liver-related morbidity and/or mortality.
- NAFLD patients have an elevated risk of liver-related morbidity/mortality and metabolic comorbidities, which place a strain on healthcare systems.
- NAFLD warrants that primary-care physicians, specialists, and health policymakers stress prevention of excessive weight gain during childhood.
- Bariatric surgery may be an alternative option to committed weight loss.
- Older age, being male, and HA are independent risk factors for NAFLD/NASH.
- NAFLD is linked with higher BMI, higher HTN, and lower physical activity.
- MetS as currently defined is not a good predictor of NAFLD in non-Hispanic blacks (NHB); because in contrast to others, TG level is normal in this group.
- Proton magnetic resonance spectroscopy is currently the best proven alternative tool to biopsy for accurate diagnosis of NAFLD.
- Treatment options require more robust studies on etiology of NAFLD.
- There is no proven medical therapy for NASH.
- Most effective therapeutic strategies include lifestyle changes including diet, exercise, modifying metabolic risk factors, early screening, and intervention.
- Certain genes may be associated with disparities in lipid metabolism.

- Alternative noninvasive markers of NASH may now be available even though there are no proven biomarkers for various stages of the NAFLD spectrum.
- Discovery of new biomolecules during clinical trials and metabolomics studies is crucial for understanding NAFLD/NASH initiation and progression.
- Patients with NASH have a worse prognosis and must be included in clinical trials of new treatments.
- The biological heterogeneity of HCC makes it difficult to assess the key mechanisms of cancer development and thus implement effective therapies.
- Certain genes have been identified to be associated with progression to HCC.

Acknowledgements

This work was supported by the National Institutes of Health (NIH) Grant U01CA185188.

Conflicts of interest

The author has no conflict of interest.

Abbreviations

AA	African-American
AF	advanced fibrosis
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index
CDC	Centers for Disease Control and Prevention
DAA	direct-acting antiviral
EA	European-American
ESLD	end-stage liver disease
HA	Hispanic-American
HCC	hepatocellular carcinoma
HDL	high-density lipoprotein
HTN	hypertension
IR	insulin resistance
LT	liver transplantation
MetS	metabolic syndrome
NAFL	nonalcoholic fatty liver
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NFS	NAFLD fibrosis score
NAS	NAFLD activity score
NHANES	National Health and Nutrition Examination Survey
NFS	NAFLD fibrosis score
T2DM	type 2 diabetes mellitus
TG	triglyceride
TCGA	the cancer genome atlas

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